SERUM ZINC AND IRON LEVELS IN CHILDREN WITH FEBRILE SEIZURES

Dissertation submitted to

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY

In fulfilment of the regulations for the award of the degree

M.D. PEDIATRICS

DR. KODALI KIRTICHANDRA

DEPARTMENT OF PEDIATRICS

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY

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GUIDE: DR. A.M.VIJAYA LAKSHMI MBBS, MD, DCH

DEPARTMENT OF PEDIATRICS

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH
THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY
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CERTIFICATE

This is to certify that the thesis entitled **"SERUM ZINC AND IRON LEVELS IN CHILDREN WITH FEBRILE SEIZURES"** is a bonafide work of **Dr. KODALI KIRTICHANDRA**done under the direct guidance and supervision of **Dr. A.M.VIJAYA LAKSHMI**in the Department of pediatrics, PSG Institute of Medical Sciences and Research, Coimbatore in fulfilment of the regulations of DR. MGR Medical University for the award of M.D degree in PEDIATRICS.

DR. A.M.VIJAYALAKSHMI
Professor,
Department of Pediatrics.

DR. S.RAMALINGAM
Principal

DECLARATION

I hereby declare that this dissertation entitled **SERUM ZINC AND IRON LEVELS IN CHILDREN WITH FEBRILE SEIZURES**was prepared by me under the direct guidance and supervision of my Professor **Dr. A.M.VIJAYALAKSHMI,** PSG Institute of Medical Sciences & Research, Coimbatore.

This dissertation is submitted to the Tamil Nadu DR. MGR Medical University in fulfilment of the University regulations for the award of MD Degree in PEDIATRICS. This dissertation has not been submitted for the award of any otherDegree or Diploma.

DR.KODALI KIRTICHANDRA

CERTIFICATE BY THE GUIDE

This is to certify that the thesis entitled **"SERUM ZINC AND IRON LEVELS IN CHILDREN WITH FEBRILE SEIZURES"**is a bonafide work of **Dr. Kodali Kirtichandra**done under my direct guidance and supervision in the Department of Pediatrics, PSG Institute of Medical Sciences and Research, Coimbatore in fulfilment of the regulations of DR. MGR Medical University for the award of M.D degree in Pediatrics.

DR. A.M.VIJAYALAKSHMI

Professor

Department of Pediatrics

CERTIFICATE BY THE H.O.D. AND PRINCIPAL

This is to certify that the thesis entitled **"SERUM ZINC AND IRON LEVELS IN CHILDREN WITH FEBRILE SEIZURES"** is a bonafide work of **Dr. Kodali Kirtichandra,**done under guidance of Prof. Dr. A.M.Vijayalakshmi, Department of PEDIATRICS, PSG Institute of Medical Sciences and Research, Coimbatore .

Dr.John Matthai **Dr.Ramalingam**

HEAD OF DEPARTMENT **PRINCIPAL**

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DR.KODALI KIRTICHANDRA

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June 26, 2013

To
Dr Kodali Kirtichandra
Postgraduate
Department of Paediatrics
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The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 21st June, 2013 in its expedited review meeting held at College Council Room, PSG IMS&R, between 2.00 pm and 3.30 pm, and discussed your study proposal entitled:

"Estimation of serum zinc and iron levels in children with simple febrile seizures"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent Form
4. Parental Consent Form
5. Data Collection Tool
6. Budget
7. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes
Dr Y S Sivan	Ph D	Member – Social Scientist	Male	Yes	Yes

The approval is valid for one year.

PSG Institute of Medical Sciences & Research
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We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Yours truly,

20.6.17

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

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INSTITUTIONAL HUMAN ETHICS COMMITTEE

CONTENTS

ABSTRACT

Introduction:

Febrile convulsions are one of the common paediatric emergencies encountered around the world. Febrile seizures occur in 6 months to 5 years of age group. Incidence of febrile convulsions around the world is between 3-4%. It is of similar incidence all over the world.Various risk factors were proposed for development of febrile seizures like developmental delay, >30 days of NICU stay, febrile convulsion history in the family, viral infections, iron and zinc deficiencies. Role of Zinc and iron deficiency in febrile convulsions were studied in western countries. But in India, there are very few studies. In this study we are proposing that low Zinc and Iron levels may predispose to development of febrile seizures.

Aim: To estimate serum Zinc and iron levels in children with febrile seizures and compare this values with febrile children without seizures.

Materials and Methods:

It is a case control study. The study group includes 50 cases (febrile seizures) and 50 controls (fever without seizures) aged 6months to 5 years (6-60 months) attending paediatric out-patient department or admitted in PSG hospital who fulfilled the inclusion criteria. Children who are having atypical febrile seizures, intracranial infections, on Zinc and iron supplementation, electrolyte and metabolic disorders were excluded from the study. Informed and written

consent was obtained from cases and controls. Temperature was recorded in Fahrenheit by using digital thermometer. 3-5ml of blood was collected from peripheral line in a red colour container. Serum was separated by centrifugation and stored at -20°C until serum zinc and iron levels were measured. Zinc and Iron levels were estimated by Atomic Absorption Spectroscopy in Stanes laboratory, Coimbatore.Categorization of socioeconomic status was done by modified Kuppuswamy scale, 2012.Nutritional status of all cases and controls were assessed according to IAP classification of malnutrition. Data was analysed using SPSS software. Methods like Chisquare and T-test were used.

Results:

Mean serum Zinc, Iron, haemoglobin, MCV, MCH, RDW levels were significantly low in cases when compared with controls (p-value <0.0001).Mean serum zinc was almost similar in <24 months and >24 months age group. Mean serum Iron and other red cell indices were low in <24 months group when compared with >24 months age group. Odds ratio was 68.3 if patient having both Iron and zinc deficiency.

Conclusions: Iron and zinc levels were significantly low in children with febrile seizures when compared to febrile children without seizures.Serum Iron was very much low in children < 2years of age when compared to > 2years of age.There is no age wise difference in mean Zinc levels. There was 68.3 times

increased risk of developing seizures, if they were having both low Iron and Zinc levels.

Key words: *Febrile seizures, Hemoglobin, MCV, MCHC, RDW, WHO, India, Iron deficiency, Risk. Zinc deficiency*

INTRODUCTION

Febrile convulsions are one of the common paediatric emergencies encountered around the world. Febrile seizures occur in 6 months to 5 years of age group. According to American Academy of Paediatrics, febrile convulsions is defined as febrile seizures occur in the absence of intracranial infection, metabolic disturbance or history of afebrile seizures, and are classified as simple or complex[1,2].

Incidence of febrile convulsions around the world is between 3-4%. It is of similar incidence all over the world. At least 3-4 % children may have one episode below 5years of age. In India, incidence is almost 10% according to some studies. But recent studies indicate that incidence is almost comparable to western population[3].

Aetiology of febrile seizures was still unclear. Various hypotheses like genetic susceptibility, hyperthermia induced convulsions were proposed. Still the pathogenesis remains inconclusive. Various risk factors were proposed for development of febrile seizures like developmental delay, >30 days of NICU stay, febrile convulsion history in the family, viral infections, iron and zinc deficiencies[1,4].

In brain, zinc is present in large quantities in the hippocampus. Zinc regulates glutamic acid decarboxylase activity which is an important enzyme in

production of γ- amino butyric acid. It also regulates the neurotransmitter affinity. It mediates inhibition of calcium on N-methyl-D-aspartate receptors there by reducing excitatory discharge of neurons. In deficiency of zinc, these receptors get stimulated which may produce epileptiform discharges in children with fever[5].

Zinc also activates pyridoxal kinase, which in turn helps in the pyrioxal phosphate synthesis from pyridoxal. Pyridoxal phosphate inturn activates glutamic acid decarboxylase which involved in synthesis of GABA. Post synaptic receptors in interaction with zinc assists in GABA action. Hence hypozincemia leads to decrease in GABA level which leads to development of seizures[5].

Iron is an important element for metabolism in the brain. It also helps in neuro transmitter metabolism. Deficiency of iron acts as an important factor in development of febrile seizures[6]. Role of Zinc and iron deficiency in febrile convulsions were studied in western countries. But in India, there are very few studies. In this study we are proposing that low Zinc and Iron levels may predispose to development of febrile seizures.

AIMS AND OBJECTIVES

- To estimate serum Zinc and iron levels in children with febrile seizures.
- To compare this values with febrile children without seizures.

Inclusion Criteria:

1) Children between 6months to 5years.
2) Seizure occurs within 24 hrs of fever and lasts for < 15mins.
3) Generalized seizures.
4) No post ictal deficit.

Exclusion Criteria:

1) Atypical febrile seizures.
2) Children with documented intracranial infections.
3) Children on zinc and iron supplementation for therapeutic purpose.
4) Electrolyte imbalance.
5) Hereditary metabolic disorders.
6) Structural brain lesions.
7) Children with cerebral palsy,mental retardation and,neuro degenerative disorders.

MATERIAL AND METHODS

The study group includes 50 cases (febrile seizures) and 50 controls (fever without seizures) aged 6months to 5 years (6-60 months) attending paediatric out-patient department or admitted in PSG hospital who fulfilled the inclusion criteria. Children who are having atypical febrile seizures, intracranial infections, on Zinc and iron supplementation, electrolyte and metabolic disorders were excluded from the study. Informed and written consent was obtained from cases and controls. Temperature was recorded in Fahrenheit by using digital thermometer. All these children have normal central nervous system examination. Complete blood count was done in all patients. 3-5ml of blood was collected from peripheral line in a red colour container. Serum was separated by centrifugation and stored at -20°C until serum zinc and iron levels were measured. Zinc and Iron levels were estimated by Atomic Absorption Spectroscopy (Agilent Technologies 200 series AA) in Stanes laboratory, Coimbatore.

Sociodemographic factors like occupation, family monthly income, and educational status were collected from parents. Categorization of socioeconomic status was done by modified Kuppuswamy scale, 2012.

MODIFIED KUPPUSWAMY SCALE:

EDUCATION

Profession or honors	7
Graduate or post graduate	6
Intermediate or post high school diploma	5
High school certificate	4
Middle school certificate	3
Primary school certificate	2
Illiterate	1

OCCUPATION

Profession	10
Semi-profession	6
Clerical, shop owner, Farmer	5
Skilled worker	4
Semi-skilled worker	3
Unskilled worker	2
Unemployed	1

FAMILY INCOME PER MONTH (in Rs) 2012 June

>31507	12
15754 – 31506	10
11817 - 15753	6
7878 – 11816	4
4727 – 7877	3
1590 – 4726	2
<1589	1

TOTAL SCORE	**SOCIO ECONOMIC CLASS**
26-29	Upper (I)
16-25	Upper middle (II)
11-15	Lower middle (III)
5-10	Upper lower (IV)
<5	Lower (V)

Weight and height were measured from cases and controls. Nutritional status of all cases and controls were assessed according to IAP classification of malnutrition[7]. Data was analysed using SPSS software. Methods like Chisquare and T-test were used.

FIGURE 1:

INDIAN ACADEMY OF PEDIATRICS (IAP) CLASSIFICATION

Normal	>80% of expected
First degree	71- 80% of expected
Second degree	61-70% of expected
Third degree	51-60% of expected
Fourth degree	< 50% of expected

Study area: PSG Hospitals, Coimbatore.

Study design: Case control study.

Sample size:50 cases and 50 controls.

Withan expected correlation between serum Zinc and Iron levels with febrile seizures as (r = -0.86) reference (LusianaMargaretha, NurhayatiMasloman, Correlation between serum zinc level and simple febrile seizure in children. PaediatrIndones 2010;50(6):326-30). And with α = 0.05 and 80% power, the sample size required is n = 9. With 20% non-response,Therequired sample size in each arm as 12.

Study population: Children aged between 6 months to 60 months (5 years) with fever with or without seizures.

Analysis of Zinc in Plasma by Atomic Absorption Spectroscopy (Agilent Technologies 200 series AA:

Figure 2:

Blood Collection and Processing of Samples:

Collected1mL of whole blood by venepuncture, using all-plastic polyethylene syringes and stainless steel needles with polypropylene infusion set shown to contribute non-detectable amounts of zinc. Then two drops (50 µL) of a 300 g/L sodium citrate solution was added into the centrifuge tubes before collecting the

specimen of blood. Centrifuged the blood immediately at 1000 x g for 15 min, and transferred it. Separated the plasma from the cells and transferred, into polyethylene storage vials, taking care to prevent disruption of the buffy coat or packed cells, because these cells can introduce relatively high amounts of cellular zinc into the plasma and hence all haemolysed samples was discarded. The samples were stored at -20°C.

Reagents and Materials:

1.Standards : Zinc standards (Merck solution traceable to SRM from NIST at 0.2-1ug/ml were prepared by diluting the stock standard solution, for zinc, with 5% (v/v) glycerol and the same 5% (v/v) glycerol solution was used as a blank solution when determining zinc. The working standards, 100, 200, 300 and 400 µg of zinc per litre were prepared. Delivered 1 mL of 1000 mg/L zinc standard into a 100 mL volumetric flask and dilute to volume with glycerol/water solution (95:1) and it was mixed by inverting. Placed the aliquots of this intermediate stock (1, 2,3, and 4mL) into four 100 mL volumetric flasks and diluted to volume with the glycerol/water mixture. The standards (0.1, 0.2, 0.3 and 0.4mg of zinc per litre) corresponded, to apparent plasma zinc concentrations of 500, 1000, 1500 and 2000 µg of zinc per litre. We shouldpreparea working curve from fresh standards, before each test. Calculatethe concentration of zinc in the plasma directly from the curve.

2. Glycerol solution: Diluted 50mL of glycerol to 1000 mL with de-ionized water. Glycerol, certified ACS (99.4%), was Fisher Scientific Co., and all volumetric glassware must meet NBS Class A specifications. Glassware and Pasteur pipettes are acid washed, soaked in disodium ethylenediaminetetraacetate (EDTA) solution (1%) for 24 h and rinsed with de-ionized water.

3. Serological pipettes and tubes: The disposable serological pipettes and polystyrene tubes used in this study contributed no detectable zinc to the sample and therefore required no pre-treatment.

4. Pooled plasma for quality control: Pooled plasma can serve as a reference to monitor inter-day reproducibility. Plasma is ideally obtained as a large single specimen from a normal healthy individual. Control plasma should be negative for and free of haemolysis. We should storein 1mL portions in individual polyethylene vials at -20°C. Thawed an aliquot at room temperature and analyzed it with the plasma samples.

5**. Sample preparation for analysis:** For the determination plasma zinc, the samples were diluted in the ratio of 1:5 with deionized water

6. **Methodology:** For The determination of zinc in blood plasma samples diluted with deionized water was carried out after appropriate dilution with a minimum of 0.2-1.0 mL serum sample, with an equal volume of a 20% (w/v)

TCA solution. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples.

Allowed the plasma samples to come to room temperature and then mixed each sample by gently inverting the tube six times. Prepare working standards as previously described. Deliver 0.5 mL of plasma sample with a serological pipette into a 16 mm plastic test tube. Add 2.0 mL of de-ionized water and immediately mix the solution for 30s. Repeat for plasma samples in groups of 10. Similarly prepare a control sample of pooled plasma.

The instrumental and gas-flow settings and aspiration rate is set precisely, to optimize signal and minimize background noise. The instrumental settings shown apply to the instrument, used in this study. Once the aspiration rate is optimized with 10 mL aliquots of water, the nebulizer flow adjustment is locked in place. Aspirated glycerol/water solution (5/95 by vol) into the luminescent flame and the baseline was set to read 0.000±0.001 absorbance (A). The baseline reading was taken before and after each sample and the baseline was reset as required.

The zinc working standards was sampled sequentially from most dilute to most concentrated, aspirating until the reading is stable (±0.001 absorbance); then record six successive 1-s integration readings. Average the readings for each sample; the resulting values are used to establish the working curve, preferably

by use of a regression least squares fit. Mix again and aspirate standardized pooled plasma sample. Calculate the concentration from absorbance readings by interpolation from the working curve. Results must be within 20µg/L (ca.3%) and the mean was previously established.

Normal zinc levels are 60-120µg/dl.

Analysis of Iron in Plasma by Atomic Absorption Spectroscopy (Agilent Technologies 200 series AA):

The above methodology was adopted for analysing Iron.

Sample Preparation: To estimate the total serum iron, samples were diluted 1:2 with a 20% (w/v) trichloroacetic acid solution, and heated. This procedure precipitates the plasma protein and removes approximately 95% of any haemoglobin iron present. Diluted 0.2- 0.5 mL of serum sample with an equal volume of a 20% (w/v) TCA solution in a polyethylene tube,. The tube was capped loosely, mixed and heated in a heating block at 90°C for 15 minutes. The sample was cooled and centrifuged. The haemolysed samples are generally discarded, even though the TCA removes about 95% of haemoglobin iron.Iron standards (Merck solution traceable to SRM from NIST at 0.5-1ug/ml) standards are prepared by diluting the iron stock solution with 10% (w/v) TCA. A 10% (w/v) TCA solution was used for the blank. Since the samples are diluted 1:2 with TCA, the instrument was calibrated to read as dilution factor x the actual concentration of the standards, so as to be able to read concentration directly. Normal iron levels were 65-120µg/dl.

REVIEW OF LITERATURE

Febrile seizures:

History:

In 460-370 BC, Hippocrates wrote that, seizures are commonly seen in the presence of acute onset of fever. He also described that seizures will be present up to 7^{th} year of life. Children crossed 7^{th} year of life and adults are not susceptible to convulsions, until there are other symptoms. He described that brain is important site of these seizures. he also described that many patients suffering from this disease will be safe but with very little damage[2]

Until 1950^{s}, they were not identified as separate entity different from epilepsies. Livingstone classified this condition into fever triggered epilepsy and simple febrile seizures. He defined fever triggered epilepsy as “febrile seizures that were of focal or of longer duration and also having family history of epilepsy”[8].

Terminology

Epilepsy[9]**:**

Epileptic seizure is a transient occurrence of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain

Status epilepticus[9]**:**

A single seizure lasting more than 30 minute duration or a series of epileptic seizure during which function is not regained between ictal events in a > 30 min period.

Definition of febrile seizure[3]:

According to National institute of health, febrile convulsions is defined as an event occurs in infancy or childhood occurring between 1 month and 5 years of age, associated with fever but without evidence of intracranial infection (USA 1980).

According to international league against epilepsy, febrile convulsion is defined as convulsion occurring between 1 month and 5 years of age, associated with a febrile illness not caused by an infection of CNS without previous neonatal seizures or a previous unprovoked seizure and not meeting criteria for other acute symptomatic seizures (1993)

American academy of pediatrics definition[1]

According to Americsn academy of paediatrics, febrile seizures occur in the absence of intracranial infection, netabolic disturbance or history of afebrile seizures, and are classified as simple or complex.

Epidemiology

In childhood, febrile convulsions are very common. Incidence of febrile seizures in United States of America and European countries is 2-4%[1,2]. According to previous studies, febrile seizure was seen in almost 10% of Indian children. But current studies showed similar incidence rates when compared to USA and Europe[3].

Age:

Febrile convulsions are most commonly occurs between 6months to 5years. Age of onset of febrile seizures in most of the cases is 18 months. Almost ½ of the febrile convulsion patients, onset of seizures is between 1- 2 years[4]. Recurrent febrile convulsions are seen in those initial seizure occurs < 1 year of age. Above 6 years and below 6months, chance of febrile seizure is very minimal or negligible[1].

Relationship between incidence of febrile seizures and age is improperly understood. In younger age group, brain is comparatively immature. Hence in response to fever, there will be increased excitability of neurons which may lead to febrile seizure[10].

Sex:

Girls are less commonly affected than boys. In almost all studies Male/Female ratio is ranging from 1.1:1 to 4:1[10].

Maternal risk factors:

Berg described that smoking and gastroenteritis in pregnancy are important risk factors for febrile convulsions[11]. In contrary a case control study conducted in Sweden which was community based showed no significant increase in occurrence of febrile seizures in the presence of antenatal or maternal risk factors[10].

Perinatal risk factors:

According to some studies, complications like prolonged labour and perinatal asphyxia, prematurity implicated as important factors in occurrence of febrile convulsions.[11]

According to a study conducted by Zwaini et al, perinatal asphyxia or gestational age were not considered as risk factor for febrile seizures. Many studies proved that NICU stay of more than 30 days considered as significant factor for development of febrile convulsion[10].

Family history:

It is already an established entity that febrile convulsions run in families. Both parents can transmit this to their children. In children with febrile convulsions, 25-40% will have strong family history of febrile convulsion. In siblings, frequency ranges between 9-22%. According to various studies, risk of developing febrile convulsion doubles, if both parents had febrile seizures rather

than single parent[3]. In twin studies, for monozygotic twins concordance rate was 35% and for dizygotic twins it is 15%[12].

1 out of 33 children in the population may have febrile seizures. After an effected child, risk of occurring in next sibling is 20% (1 out of 5). Risk increases to 1 out of 3, if previous child and both mother and father had history of febrile seizures. Only one out of ten may proceed to afebrile seizures which are recurrent[13,14]. Many studies concluded that inheritance for febrile seizure susceptibility is polygenic and very rarely autosomal dominant[3,15].

Genetic factors:

Various models have been proposed. Universally accepted models are polygenic, autosomal dominant, and a multifactorial model. Complex segregation analysis was done by Rich et al through febrile seizures pro-bands in 467 families. They confirmed that polygenic model of inheritance was seen in these families with single episode of febrile seizure[15].

In families with multiple febrile seizure episodes, model proposed was single major locus model. In families with 52 febrile seizure pro-bands, Johnson et al. conducted a pedigree study. They confirmed that autosomal dominance with decreased penetrance was seen in these families[15,16].

Febrile seizure phenotypes[15]**:**

Recently Scheffer and Berkovic and Singh et al. termed a subset called GEFS+ (generalised epilepsy with febrile seizures plus). In this mode of inheritance is autosomal dominance. The term GEFS+ was used when there is continuation of fever with seizures in children > 6 years/ associated with non-febrile generalised tonic clonic seizures. This was self-limiting condition which was resolved by middle of adolescence.

Phenotype spectrum (Figure 3)

Febrile seizures
Febrile seizures+
Febrile seizures+ with other seizure types (absences, myoclonic, or atonic seizures)
Myoclonic- astatic epilepsy

There isassumption that febrile seizures and epilepsy were two different entities. But there is considerable overlap in genetics and clinical features between febrile seizures and epilepsy. It is possible that susceptible to febrile seizures gives rise to epilepsy in later stages of life.

Linkage mapping studies[15,17–19]:

By various studies, loci for febrile seizures were identified. They are FEB 1, 2, 3, 4, and GEFS+ locus.

Figure 4: Febrile seizure loci

FEB 1 (febrile convulsions, familial, 1: NIM602476)	Wallace et al. identified a locus on 8q13-q21 for an autosomal dominant familial febrile seizures.
FEB 2 (febrile convulsions, familial, 2: NIM602477)	Johnson et al. identified an autosomal dominant febrile seizure locus on 19p13.3.
FEB 3 (febrile convulsions, familial, 3: NIM604403)	Peiffer et al. identified an autosomal dominant FS locus on 2q23-q24.
FEB 4 (febrile convulsions, familial, 4: NIM604352)	Iwasaki et al. identified a locus on 5q14-q15 conferring susceptibility to febrile seizures
GEFSP1 (generalised epilepsy with febrile seizures+ Type 1: MIN 604235)	Wallace et al. found linkage to 19q13.1 in large GEFS+ family. They also identified a mutation, Cys121Try in the SCN1B gene.
GEFSP2 (generalised epilepsy with febrile seizures+ type 2: MIN 604233)	Baulac et al. identified a GEFS+ locus on 2q21-q33. Escayg et al. described two mutations of the gene encoding SCN1A gene, Thr875Met and Arg1648His.

Immunisation:

Three vaccines are identified as risk factors for febrile convulsions. They are MMR vaccine, DPT vaccine, and Influenza vaccine.

MMR vaccine:

Following MMR vaccination, there is transient risk of febrile seizures when compared with those who are not vaccinated. The occurrence of febrile seizures could be due to fever induced by vaccine. Vestergaard and Hviid et al. conducted a large cohort study in Denmark. Out of 439251children who received vaccination, 17986 children had single febrile seizure episode. Within 2 weeks of vaccination, 973 out of 17986 children had febrile seizures[20]. Griffin et al. found that febrile seizures risk was high with in first 2 week of vaccination[21].

DPT vaccine:

DPT vaccination was related with increased risk of convulsions and encephalopathy. Within 3 days of DPT vaccination, there was increased risk of febrile seizures. Barlow et al. conducted a large scale cohort study in USA. Out of 340386 DPT vaccination and 137457 MMR vaccinations, 487 children developed febrile seizures and 137 children developed afebrile seizures. Incidence of febrile seizures following MMR and DPT vaccination found out to be 25-30 and 6-9 per 100000 children[21].

Influenza vaccine:

They found out that there is increased risk of febrile seizures between 6 months to 4 years with FLUVAX and FLUVAX junior. They found out that there are increased rates of fever within 1 day of administration. Incidence of febrile seizures after fluvax or fluvax junior found to be ≤9 per 1000. Other brands like TIV or LAIV found to be safer and can be administered. According to ACIP, Vaccines recommended in the age group between 6 months to 8 years are TIV or LAIV[22].

Viruses as a precipitating factor:

In previous studies, approximately 40 % febrile seizure children have various viral infections. Hall et al. reported that 1/3rd of the febrile seizures children have Human Herpes Virus 6 infection (HHV-6). HHV-6 found to be an important factor for recurrent episodes. Van zeijl et al. reported that risk of recurrence is more in children with influenza A. rotaviral gastroenteritis have twice the risk of convulsions when compared with other causes[23].

Chung et al. conducted a retrospective study in Hong Kong. In this study they reviewed 923 children with febrile seizures. 565 out of 923 children have admitted for first episode of seizure. They found out that 163 out of 923 (17.6%) found to have influenza infection. 63 out of 923 (6.8%) found to have adenovirus infection. 55 out of 923 (6%) found to have para influenza infection.

25 out of 923 (2.7%) found to have RSV infection. 12 out of 923 (1.3%) found to have rotavirus infection. Incidence of influenza, para influenza, adenovirus, RSV and rotavirus in febrile convulsions were 20.8%, 20.6%, 18.4%, 5.3%, and 4.3% respectively[23].

Pathophysiology of febrile seizures:

Various animal models help in understanding of disease process. Usually these models are appropriate for age. Hyper thermic seizure model is a model in which outside heat will be used to produce hyperthermia. Heat can be produced from heat sources like microwave, heat lamp etc. In “febrile” seizures model, endotoxin lipopolysaccharide will be injected which will produce immunological reaction and fever[24].

Mechanisms of febrile seizures:

Temperature:

If febrile convulsion developed in the presence of low fever, these children have higher risk for development of further seizures. This may be because of low threshold for convulsions. GABA (A) receptor usually mediates excitation or inhibition of neurons. If temperature > 38°c, it reduce the inhibition on neurons. Hence it leads to un-opposed excitation of neurons. This may produce convulsions[24].

Mediators of inflammation:

Cytokines like tumour necrosis factor, interleukins play an important role in various neurological diseases. Helminen and Vesikari in 1990 found that an increased interleukin 1β production may play an important role in development of seizures[24]. In previous studies, they tried to control the seizures with paracetamol (antipyretics). But they found out that they are not effective. So they tried to examine alternate pathway (effect of cytokine production on development of seizures)[25].

Figure 5: Effect of cytokine production on febrile seizures

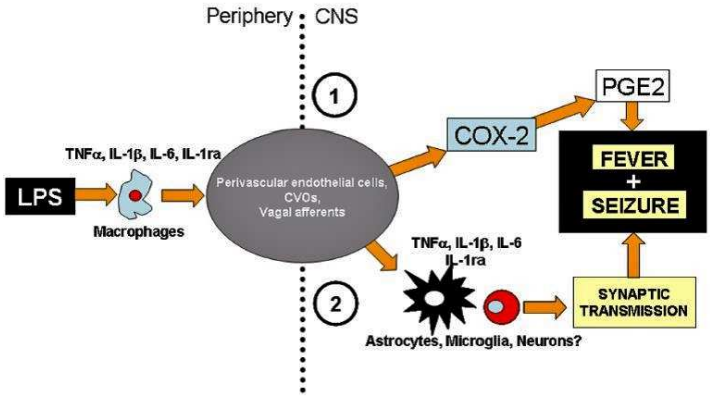


FIGURE 1.
The peripheral and central response to LPS. Peripherially LPS stimulates macrophages to secrete pro- and anti-inflammatory cytokines. These can then act through perivascular endothelial cells circumventricular organs (CVOs) or vagal afferents to stimulate COX-2. COX-2 activation increases the production of prostaglandin E2 which then causes fever (path 1). At the same time pro- (TNFα, IL-1β, and IL-6) and anti-flammatory (IL-1ra) cytokines are generated in the brain (path 2). These cytokines have been shown to modulate neurotransmission and ultimately could contribute to the generation of seizures during fever.

In cytokines, interleukin 1β was implicated in pathogenesis of febrile seizures. Seizures were produced due to changes in NMDA receptor phosphorylation by IL- 1β. Vivianietal.found that there is an increased Ca^{+2}influx. This was due to NR2A/B phosphorylation which is a subunit of NMDA receptor[25].

Respiratory alkalosis:

It is well observed fact that there is increase in respiratory rate whenever there is an increase in temperature of the body. So there may be hyperventilation in the presence of hyperthermia. In experimental rat models, it was proved that hyperventilation may lead to respiratory alkalosis. It may act as a triggering factor for development of seizures. Increased atmospheric Co2 to 5% may result in reduction of convulsions[26].

Two-Hit hypothesis:

According to various studies conducted on rats that have neuronal migration disorders, they have lesser threshold for hyper thermic convulsions. In later stages they are more prone for temporal lobe epileptic disorder. In brain with previous damage, febrile convulsions may produce serious effects than normal brain[24].

Effects of febrile seizures:

According to various studies, febrile convulsions may produce following changes.

Functional changes[24]**:**

According to large cohort study conducted in Denmark, in children with febrile convulsion history had 5times more risk of developing afebrile seizures in later part of life. But after twenty three years of follow up, <7 % had epilepsy. In other studies they reported that there is a possible relation between

febrile seizures and temporal lobe epilepsy which is classified under complex partial seizures.

In some prospective studies, they reported that large proportion of complex febrile seizures patients may develop epilepsy. But risk is still very less. Simple febrile convulsion will not have any effects on behaviour and development normally. But if it is occurred below 1st year of age, they may have delay in language and development. These children may need to join in special schools[24].

Changes in structure:

There may changes in limbic system in children with febrile seizures. MRI may show changes in the hippocampal structure. According to MRI studies done in 11 children with febrile status epilepticus, abnormal signal intensity in hippocampus seen in 7 children developed within 3 days of seizure. During follow up, 5 out of 7 children had mesial temporal sclerosis[24].

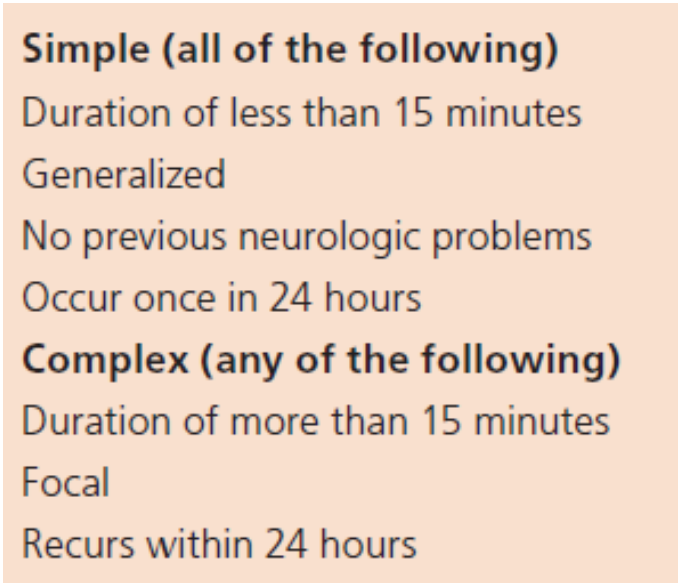
Molecular changes:

Hyper thermic seizure was known to alter benzodiazepines and GABAbinding to GABA (A). It also decrease the inhibition in the hippocampus which is GABA (B) receptor mediated[24].

Clinical features:

Febrile seizures broadly classified into simple or complex febrile seizures

Figure 6: Simple Vs complex febrile seizures



In simple febrile seizures there is no post ictal confusion or loss of consciousness, whereas in complex febrile seizures there may be post ictal confusion[1,4]. Febrile convulsions may present as initial manifestation of a fever. Convulsion can be tonic clonic or any other variety. But generalised tonic clonic seizures are very common[8].

Differential diagnosis[1,4]**:**

Figure 7:

Breath holding spells- acute reactions to noxious stimuli which are usually unexpected
Reflex anoxic seizures- acute reactions to noxious stimuli
Syncope – associated with limpness & bradycardia rather than tonic clonic movements & tachycardia
Rigors and Tetany- conscious will not be lost

Evaluation of a child with febrile convulsion:

Evaluation involves history taking, complete physical examination, and lab investigations.

History[8]**:**

1) Symptoms of various infections,
2) Drug usage,
3) H/o trauma,
4) Development level,
5) Family history of febrile or afebrile convulsion
6) Any recent immunisation history

Physical examination:[8]

1) Consciousness
2) Examination of all systems
3) Anterior fontanel – at level/bulging
4) To look for presence of meningeal irritation.
5) To look for any focal neurological abnormalities.

Lab investigations (AAP guidelines):

Simple febrile convulsions:

Routine investigations:

Routine investigations like complete blood count, urine routine and serum electrolyteswere not needed until there is suspicion of infection. Presence of bacteria in the blood is same in children <2years of age with or without convulsions. In some cases there may be electrolyte abnormalities. In those cases depends on clinical history and condition of the patient, one should decide on doing routine investigations[1,8,27].

Indications for Lumbar puncture[1,8,27]:

1) Lumbar puncture is indicated in a child having signs of meningeal irritation.

2) Any child between 6months to 1 year with deficient vaccination against haemophilus influenza and pneumococcus – lumbar puncture is considered optional.
3) Any child admitted with fever and convulsions and already on antibiotics, lumbar puncture is considered optional.

EEG (Electro Encephalogram)[1,8,27]:

In neurologically normal child with simple febrile convulsions, EEG is not indicated. In some studies EEG showed paroxysmal findings.

Neuro imaging[1,8,27]:

Neuroimaging like MRI and CT scan will not be indicated in simple febrile convulsions. CT scan is associated with exposure to radiation.

Complex febrile convulsions[1,4,28]**:**

Routine investigations:

Complete blood count and urine routine are recommended to rule out infectious aetiology. Serum electrolytes can be done if we suspect any biochemical abnormality.

EEG:

Electroencephalogram is recommended in recurrent episodes of complex febrile seizures. EEG is indicated to rule out conditions like encephalitis.

Lumbar puncture:

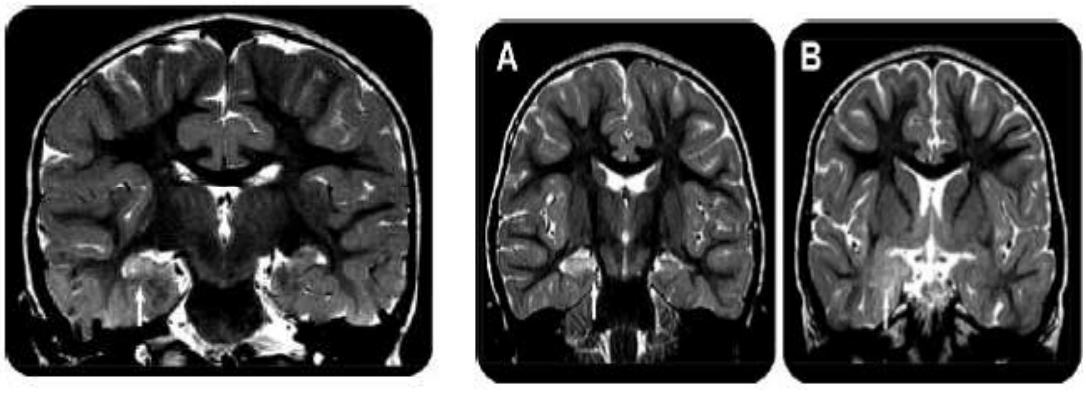
Lumbar puncture should be performed in all children with clinical suspicion of CNS infection.

Neuroimaging:

CT brain or MRI scan can be done to rule out abnormalities in the brain. Shinnar et al. conducted a FEBSTAT study in febrile status epilepticus children. They reported that hippocampal abnormalities are seen in 10.5% of the cases. 7.9% of the status epilepticus children showed extra hippocampal temporal lobe abnormalities[29].

Figure 8:

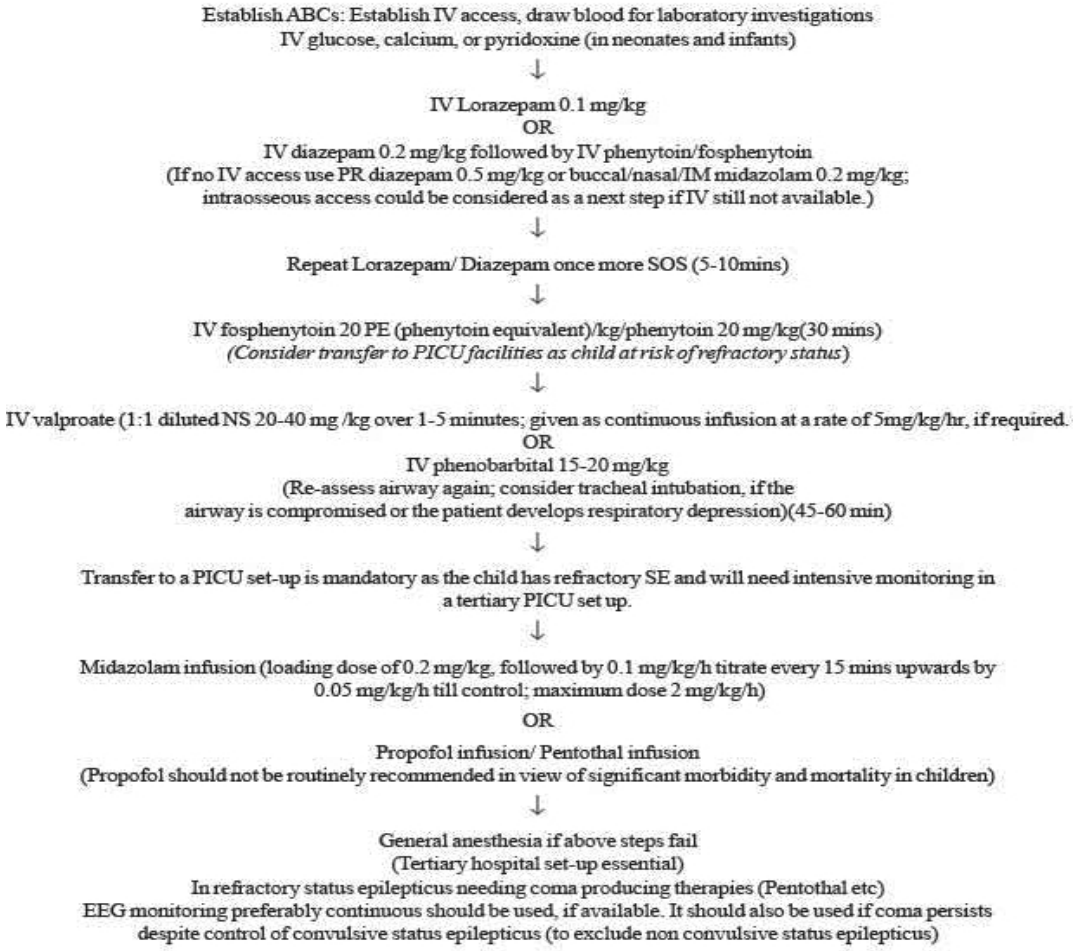
Hippocampal abnormalityExtra hippocampal abnormalities



Treatment of active seizures:

Any child arrived to EMD with seizures; we have to treat it as status epilepticus unless otherwise proven.

Figure 9: Management of active seizures



Management and prognosis for simple febrile seizures:

Management of simple febrile convulsions includes long term treatment or intermittent prophylaxis.

Long term treatment[1,4,30,31]**:**

This includes treating these children with long term anti- epileptic drugs. Various studies were conducted for effective and safer anti- epileptic to prevent further seizures.

Phenobarbital:

Camfield et al. conducted a randomised control trail in 79 children with febrile convulsions. In this they have given phenobarbital 5mg/kg/day single dose daily or placebo. They found out that recurrence rate came down significantly in phenobarbital group than placebo group.

Adverse effects are

1) Lethargy.
2) Hyperactivity.
3) Sleep disturbances.
4) Irritability.
5) Hyper sensitivity.
6) Short term memory impairment.

As adverse effects are more than benefits, AAP is not recommended the long term management with phenobarbital.

Sodium valproate:

Mamelle et al found out that phenobarbital was less effective when compared with sodium valproate. Fewer studies were done on valproic acid safety.

Adverse effects:

1) Hepatotoxicity.
2) Renal toxicity.
3) Pancreatitis.
4) Haematological problems.

There are more adverse effects than benefits. So this drug is also not recommended by AAP.

Primidone:

Various Studies found out that 15-20 mg/kg/day of primidone is effective in reducing recurrence. But irritability, behavioural problems, sleep problems also seen with primidone. Hence it is not recommended.

Carbamazepine and Phenytoin:

Phenytoin and carbamazepine are not effective in reducing the recurrence.

Intermittent prophylaxis[1,4,14,30,31]**:**

Intermittent prophylaxis includes treatment with antipyretics and anti-epileptics.

Antipyretics:

Camfield et al found out that 25% of the children had developed recurrence with only control of temperature. Schnaiderman et al reported that acetaminophen given at 15-20mg/kg/dose Q 4^{th} hourly did not prevent recurrence of seizure. AAP is not recommending the use of Antipyretics for intermittent prophylaxis.

Intermittent anti-epileptic prophylaxis:

Diazepam:

Autret et al conducted a randomised control trail. They reported that 0.2mg/kg of oral diazepam was not effective when compared with placebo group.

Adverse effects of intra nasal, oral or rectal diazepam:

1) Drowsiness
2) Lethargy
3) Respiratory depression
4) Ataxia

AAP is not recommending the use of diazepam as intermittent prophylaxis.

Clobazam:

Rose et al conducted a randomised controlled trial. They found that 1.7% of 60 patients have recurrence when compared with placebo group. They also found that weakness and drowsiness seen equally in both diazepam and clobazam group. Ataxia was comparatively lower in clobazam group. They concluded that clobazam is safe and effective than diazepam as intermittent prophylaxis[32].

Prognosis:

Febrile convulsions usually carries good prognosis. Physician should ensure parents that there are no long term neurological sequelae from febrile convulsions. In UK, they conducted a population bases study. They followed up 381 children prospectively up to 10 years of age. They found out that they perform as good as normal children in all aspects[1].

In Denmark, they conducted a cohort study in 1600000 children. They found out that there is slight increase in death rate in children with complex febrile convulsions (within 2 years). In simple febrile convulsions, there is no significant increase in death rate[1,33].

Figure 10: Risk of recurrent febrile convulsion[1,14]

Risk factors	*Number of risk factors*	*2-year risk of recurrence (%)*
Age < 18 months	0	14
Duration of fever < 1 hour before seizure onset	1	> 20
First-degree relative with febrile seizure	2	> 30
Temperature < 104°F (40°C)	3	> 60
	4	> 70

Figure 11: Risk factors for developing epilepsy[1,14]

Complex febrile seizure*
Family history of epilepsy
Fever duration < 1 hour before seizure onset
Neurodevelopmental abnormality (e.g., cerebral palsy, hydrocephalus)

**—Febrile seizures with multiple complex features are a possible risk factor.*

Zinc (Zn)

Historical aspects:

In 1869, importance of zinc was established for plants. In humans, zinc importance was established in 1961. In 1961, it was reported that an Iranian farmer developed a syndrome of short stature, hypogonadism and anemia who was taking unrefined flat bread, potatoes and milk. Similar case reports

published on Egyptian adolescents. After 1961, zinc deficiency is known as important nutrition problem in the world [34].

Zinc is an important trace element among all micronutrients. In developing countries, nearly 2 billion people are zinc deficient. Zinc deficiency causes increase in diarrhoea and infection leading to death of around 8 lakhs children in the entire world. Zinc deficiency is one of the risk factor for immunodeficiency and infection susceptibility in older people. Hence dietary intake of zinc will have impact on various aspects of human health.[35]

Sources of zinc

Major animal sources of zinc are oysters, shell fish, lobster, poultry, pork and dairy products. Predominant sources of zinc in plants are soy foods, peas, nuts, cereals which are fortified, sea vegetables, seeds, beans which are cooked and dried. Though zinc is available in large number of edible products, intake of easily absorbable zinc (i.e red meat, oysters, liver, mushrooms, crabs and poultry) foods is less in most of the developing countries[35].

Although tubers, legumes, cereals which are staple traditional foods contain zinc, the bioavailability in these products is reduced due to formation of insoluble complexes with zinc by phytate, lignin and fibres thereby decreasing its absorption. Zinc is available in negligible quantities in fruits and vegetables[35].

Figure 12: Zinc contents of various foods[36]

Typical Zinc Content (mg/100 g)	*Food Item*	
<1	Chicken breast	Vegetables: roots and tubers
	Chicken liver	Vegetables: fruits
	Tuna	Fruits
	Salmon	Tofu
	Other finfish	Eggs
	White rice	Cottage, Cheddar and Blue cheese
	Vegetables: leaves, stems and flowers	Nuts (almond, walnuts)
1–2	Dark chicken meats	Eel
	Pork loin	Shrimp
	Sword fish	Beans (Navy, Black, Pinto, etc)
	Mushrooms	Bran cereals
	Whole milk	Nuts (cashews, pecans, peanuts)
	White wheat flour/ white bread	
2–4	Veal	Bovine kidney
	Lamb	Pig kidney
	Pork	Rye kernel
	Turkey dark meat	Barley kernel
	Lobster	Oat kernel
	Clam	Buckwheat kernel
	Crab	Peanuts, roasted
	Skim milk	Lentil
	Yogurt	Whole wheat flower
	White bean	Corn meal
	Chicken pea	Some breakfast cereals
4–10	Duck	Pork
	Beef	Lamb
	Beef liver	King crab
	Pig liver	Some breakfast cereals
>10	Oyster	Breakfast cereals fortified with zinc
	Peanut butter crunch	Beef chuck and lean beef shank

Zinc metabolism

Absorption of zinc[34]

After dietary intake, it will reach the small intestine where it absorbed by carrier mediated transport. Zinc absorption depends on type of feed and amount of zinc in the food. If zinc was administered in the form of liquid solution to fasting individuals, it will be absorbed more (60-70%). Very less amount of zinc will be adsorbed from the solid diets.

Accepted average zinc absorption is 33%. Zinc absorption rate varies in different population groups due to different types of dietary practices and phytate: zinc molar ratio. Zinc status plays an important role in zinc absorption. Those individuals, whose diet contains less zinc, will absorb zinc more efficiently. Whereas individuals zinc rich diet show reduced efficiency of absorption.

During digestion, zinc is released as free ions from the food. Before their transport into enterocytes in the duodenum and jejunum, these free ions will bind to ligands (secreted endogenously). Afterwards free ions attached to various transport proteins which leads to passage of zinc into portal circulation. If zinc was taken in higher amounts, some part of it will be absorbed through passive para cellular route.

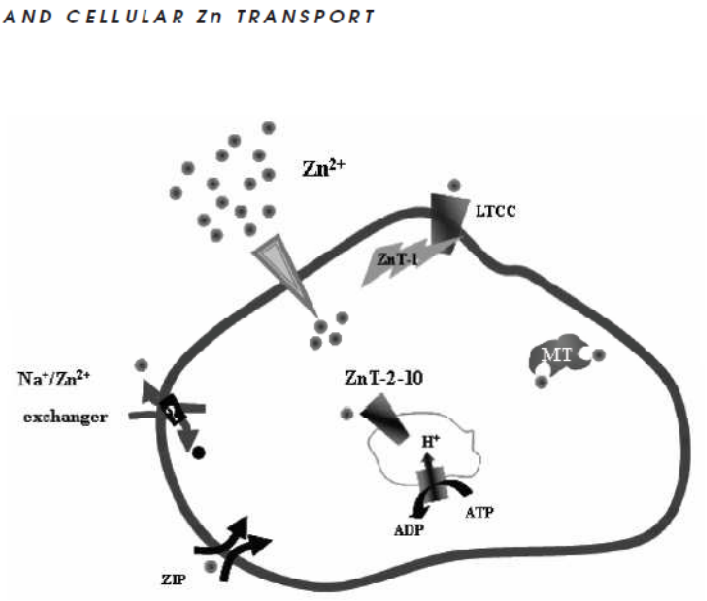
Through portal circulation, absorbed zinc reaches liver. From the liver zinc is released into systemic circulation. From systemic circulation zinc is released to various tissues. In systemic circulation, 70% of zinc is attached to albumin. If any reduce in serum albumin levels will have effect on zinc levels in the serum.

Zinc transporters (ZnTs)[34]

There are minimum 10 zinc transporters and 15 zip transporters and 3 forms of metallothionein in human cells. These transporters appear to have contrasting roles in zinc homeostasis at cellular level. Zinc transporters were regulated by changes in zinc level[6].Zinc Transporterslowersthe availability of

zinc in intracellular level by enhancing efflux of zinc from cells into intracellular vesicles whereas, zip transporters increase the availability of zinc in intracellular level by enhancing extracellular zinc uptake and vesicular zinc release into the cytoplasm.Both will exhibit tissuespecific expression, varied responsiveness to dietary zinc deficiency/ excess.[34]

Figure 13: **Active zinc transport across the cell membrane**



These gradients are produced by 2 mechanisms. (1) primary pump by using the energy of ATP- hydrolysis, (2) Na^{+}/ Zn^{2+}exchanger.[37]

(1) Primary pump

In some studies they proposed that ATPase pump actively transports across cell membrane. But still there is no evidence has been found in mammalian or human cells.

(2) Na^{+}/ Zn^{2+}exchanger ($3Na^{+}$/ $1Zn^{2+}$)

It is a member of Na^{+}/ Ca^{2+}exchanger super family which promotes efflux of Zn^{2+}against 500 fold transmembrane gradient.

Zinc homeostasis:

Primary mechanisms for maintaining Zinc homeostasis are changes in the absorption and excretion of zinc in the gastro intestinal tract and renal regulation

1) Gastro intestinal regulation:

It is the key site for regulation of homeostasis.In this, mechanism will be changes in excretion into the faeces and absorption of zinc. According to Jackson et al. (1984), with increase in intake of zinc, excretion of zinc increased. Absorption of zinc will respond more slowly to increase in intake of zinc.[38]

2) Changes in faecal excretion of zinc:

It is made up of 2 components (1) obligatory / metabolic loss, (2) endogenous loss (Weigand and Kirchgessner 1980). According to Baer and King 1984, obligatory faecal excretion of zinc was estimated from quantity of zinc excreted (approx. 6-8μmol/d). If intake of zinc reduced, efficiency of zinc absorption increased. If absorption of zinc reduced, faecal excretion of zinc also reduced.[38]

3) Renal regulation:

Renal loss of zinc will be low when compared with gastro intestinal losses. When intake of zinc is very low, urinary zinc extretion will be decreased.

4) Other sources of loss of zinc[38]:

a. Insensible losses (sweat and other surface losses)
b. Seminal emissions (approx. 9μmol per ejaculum)
c. Menstrual losses
d. Hair and nail growth (approx. 0.5μmol zinc loss/day)

Zinc is present in all body tissues with majority is present in bone and muscle (Figure 14).

Distribution of zinc within the body in a normal adult man (70 kg)[1]

Tissue	Zn concentrate	Percent of total body zinc
	μg/g wet weight	%
Skeletal muscle	51	57
Bone	100	29
Skin	32	6
Liver	58	5
Brain	11	1.5
Kidneys	55	0.7
Heart	23	0.4
Hair	150	~0.1
Blood plasma	1	~0.1

If intake of zinc was very low or only small amounts of zinc taken for long periods, homeostatic changes may not be adequate to replace losses. With

severe deficiency of zinc, whole body content reduced. But loss will not be constant throughout the body. In skin, heart, hair and muscle, zinc concentrations were almost normal. In testes, liver, bone and Plasma, zinc concentrations were decreased significantly[38].

During catabolic phase, zinc is released and taken up by various tissues.During this phase, zinc was liberated by bone and taken up by muscle tissue. In severe Zn deficiency, increased retaining of zinc in specific tissues leads to pronounced drop in internal losses of zinc[38].

With very low intakes of zinc, primary homeostatic mechanisms become inactive which leads to activation of secondary mechanisms. These includes decrease in excretion of zinc in urine, rise in fractional turnover rates, increased retaining of zinc in specific tissues such as muscle to maintain homeostasis[38].

Dietary components effecting zinc absorption

Zinc absorption from water solutions was considerably high when compared to zinc absorption from meals. Zinc bioavailability also depends on type of protein. Animal proteins curb the inhibition of zinc absorption by phytates. This effect is due to release of amino acids from proteins. According to sandstorm et al, absorption of zinc was lower in cow's milk than infant formula which is whey based. Because whey based milk has positive effect on absorption of zinc when compared to casein[39].

Staple foods like corn, cereals, legumes, rice contains phytates which have negative effect on absorption of zinc. Phytate composed of different types

of inositol phosphate like hexa phosphates, triphosphates penta phosphates and tetra phosphates. Out of which pentaphosphate and hexaphosphate have negative effect on absorption of zinc[39].

The amount of calcium in the meal will have negative effect on zinc absorption as calcium tends to form complexes with zinc and phytate. Organic acids like lactic, malic and citric acid will increase absorption of zinc[39].

Figure 15: Dietary requirements of zinc[40]

Table 3: Estimated physiological requirements for absorbed zinc by age group and sex

Variables	WHO		Variables	FNB		IZiNCG	
Age	Reference wt. (kg)	Requirement (mg/day)	Age	Reference wt. (kg)	Requirement (mg/day)	Reference wt. (kg)	Requirement (mg/day)
6-12 mo	9	0.84	6-12 mo	9	0.84	9	0.84
1-3 year	12	0.83	1-3 year	13	0.74	12	0.53
3-6 year	17	0.97	4-8 year	22	1.20	21	0.83
6-10 year	25	1.12					
10-12 year	35	1.40	8-13 year	40	2.12	38	1.53
12-15 year	48	1.82					
15-18 year M	64	1.97	14-18 year M	64	3.37	64	2.52
15-18 year F	55	1.54	14-18 year F	57	3.02	56	1.98
Pregnancy	-	2.27	Pregnancy*	-	4.1-5.0	-	2.68
Lactation	-	2.89	Lactation*	-	3.8-4.5	-	2.98

*Different stages of pregnancy/lactation. WHO=World Health Organization; FNB=Food and Nutrition Board; IZiNCG=International Zinc Nutrition Consultative Group

Functions of zinc:

Cell level functions can be of 3 categories:

Structural:

It has a principal role in cell membrane and proteins structure. So deficiency of zinc leads to increase in susceptibility of cell membranes to oxidant induced damage.

Regulatory:

Zinc proteins acts as transcription factors which controls the expression of gene. Zinc helps in release of hormones and nerve impulse propagation by sending signals to the cells.

Catalytical:

Zinc plays a pivotal role as a catalyst in over 100 enzyme mediated reactions[41].

Zinc deficiency

Epidemiology

World:

It plays a considerable role in global anemia burden. In countries where populations taking very low quantities of animal sources and plant sources with excessive inhibitors like phytates are at potential risk of deficiency. According to world health organisation (WHO) estimates, 1/3rd of the world population (approx. 2 billion people) have deficiency of zinc. Deficiency of zinc found out to be important risk factor for pneumonia and diarrhea which contributes to 20% perinatal mortality rate throughout the world. Zinc is a risk factor in 18% of malarial cases, 16% of LRTIs, 10% of diarrheal cases throughout the world[42].

High risk groups:

1) Infants and children:

Due to rise in requirements for growth, this group is at higher risk of developing deficiency of zinc. Infants who are fed only with breast milk will have adequate requirements up to 6months of life. After that infants started on weaning which should contain adequate quantity of zinc to maintain whole body zinc. Deficiency of zinc in poor countries is due to delay in starting of weaning or cereals with low quantity of zinc.

In low birth weight babies, there will be very low stores of zinc in the liver. In preterm infants, zinc levels still reduced as zinc will be transferred in later part of pregnancy. Due to immature GIT, Premature babies will have decreased absorption. Requirements of zinc in undernourished children will be higher than normal children.

2) Adolescents:

During puberty, physiological zinc requirements will be higher when compared to other groups.

3) Pregnant and lactating woman:

Due to increased caloric requirement, this group tends to develop deficiency of zinc. During lactation, requirement of zinc is even higher[7].

4) Elderly:

Intake of zinc in old people is low according to various diet surveys. Absorption of zinc will be decreased with increase in age[34].

Figure 16: Causes of deficiency of zinc[43]

1. Inadequate intake 1) Low-zinc-containing diets: Foods poor in animal protein (vegetarians) 2) Loss of zinc during food processing (desalting during production of artificial milk) 3) Prolonged intravenous alimentation, enteral alimentation 4) Shortage of nutrient intake 2. Malabsorption 1) Congenital: Acrodermatitis enteropathica (very rare) 2) Acquired (1) Ingestion of absorption inhibitors: Phytic acid, edible fibers (2) Malabsorption syndrome: Liver dysfunction, pancreatic dysfunction, inflammatory bowel disease, short bowel syndrome (3) Drugs, chelating agents: EDTA, penicillamine	3. Excessive loss 1) Loss into digestive fluid: Child intractable diarrhea, intestinal fistula, gastrointestinal disease associated with diarrhea 2) Increased urinary elimination: Liver cirrhosis, diabetes mellitus, renal disease, hemolytic anemia, intravenous alimentation, enhanced catabolism (surgery, trauma, infection, etc.), diuretics, sodium polyphosphate 3) Others: Burns, hemodialysis 4. Increased demand Pregnancy, neonates (premature babies), enhanced anabolism (during intravenous alimentation, etc.) 5. Unexplained Congenital thymus defect, Mongolism

*Underlined = particularly important

Figure 17: Symptoms of deficiency of zinc[43]

Anorexia	Hypogeusia/Hyposmia
Growth retardation	Pica
Skin symptoms	Depression/Emotional instability
· Extension from mucocutaneous junctions (mouth, eyes, anus, etc.) to the periphery	Ataxia
	Dementia (hypothesis)
· Bullous or pustular dermatitis, erosive eczema, hyperkeratosis, skin atrophy	Reduced glucose tolerance
	Increased incidence of cataracts
Alopecia/baldness	Disturbed dark adaptation (night blindness)
Gonadal hypofunction	Increased incidence of ischemic heart disease
Delayed wound healing	Increased carcinogenesis
Susceptibility to infections (compromised immune function)	Abnormal pregnancy

Deficiency of zinc divided into mild, moderate and severe. In mild deficiency, features like decreased taste sensation, decreased count of sperms, low testosterone levels, loss of weight. In moderate deficiency which is generally associated with malnutrition and chronic diseases, usually presents as growth retardation, late development of gonads, abnormalities of skin, poor appetite, excessive sleepiness, decreased adaptation in dark environment, late healing of wounds. In severe deficiency, it is presented with acrodermatitisenteropathica, pustular / bullous dermatitis, diarrhea, mental depression, alopecia. Deficiency of zinc also causes repeated infections[43].

Role of zinc in various diseases:

Diarrhea:

Zinc is important micronutrient which prevents damage by oxidants. As loss of zinc through diarrhea, deficiency of zinc is usually associated it. Hence supplementation of zinc became important mode of intervention in management of diarrheal disease. Many studies showed beneficial effects of zinc in diarrheal disease and its role in prevention[7,44].

Out of these studies, many studies were done in Asian countries. In these countries, deficiency of zinc is common. According to combined data from various randomized controlled trials on role of zinc in diarrhea, those who received supplements of zinc showed 15% reduced likelihood of persistence of diarrhea when compared with control group. In persistent diarrhea patients,

there will be 24% reduced likelihood of continuing diarrhea. If given supplements of zinc, there will be lower failure of treatment and death[7].

According to Bhatnagar S et al, those children who were supplemented with zinc, output of stools decreased by 31% than in control (placebo) group. These findings will not vary with nutrient status or age. Effect of zinc on diarrhea will not vary depending on the type of salt used[7].

Pneumonia:

Pneumonia is important cause of mortality all over the world. Zinc has important role in immunity. According to Bhandari N et al, supplementation of zinc found to have 26% reduction in pneumonia [45]. According to brooks et al, supplementation of zinc 30% decrease in period of severe respiratory infection and there was 25% average reduced stay in the hospital[46].

Malaria:

According to Shankar AH et al, supplementation of zinc observed to decrease visits to hospitals for malaria. According to Vmuller et al, there will not be significant effect of supplementation of zinc on malaria. According to zinc against plasmodium group, there is no significant effect on malaria even after improvement in zinc levels by supplementation of zinc[47–49].

Role of zinc on mortality:

According to Baqui AH et al, children who received supplementation of zinc reduced duration of diarrhea and incidence when compared with controlled group. There is also reduced admission to hospital and mortality due to diarrhea[50].

According to Sazawal S et al, supplementation of zinc in low birth weight babies leads to 68% decrease in death. These babies were supplemented with 5mg of zinc daily from1month of age to 9months of age[51].

Role of zinc on growth and development[52]:

Zinc was involved in metabolism of bone. Hence zinc has very crucial role in development. Zinc also important part bone matrix. Through its positive effect on synthesis of DNA, it increases the effect of vitamin D.

Maternal deficiency of zinc leads to various effects on the baby.

Figure 18:

CONSEQUENCES OF MATERNAL ZINC DEFICIENCY
Spontaneous abortion
Congenital malformations
Low birth weight intrauterine growth retardation
Preterm and post-term delivery
Prolonged or inefficient first-stage labor
Protracted second-stage labor
Premature rupture of membranes
Pregnancy-related toxemia

Supplementation of zinc will reduce loss of appetite which in turn beneficial for growth. Zinc activates insulin like growth factor which in turn helps in growth of the individual[52].

Role of zinc in brain and behavioural function:

Zinc plays important role in CNS development. Deficiency of zinc can result in restriction in development of intellectual functions. Various enzymes which are dependent on zinc helps in growth of the brain. Zinc proteins take part in neurotransmission and structure of brain. Various neuro transmitters which are dependent on zinc take part in functions like memory. Zinc also took part in manufacturing of precursor neurotransmitters[52]

Role of zinc in febrile seizures:

In brain, zinc is present in large quantities in the hippocampus (approx. 30μg/g weight). Zinc regulates glutamic acid decarboxylase activity which is an important enzyme in production of γ- amino butyric acid. It also regulates the neurotransmitter affinity. It mediates inhibition of calcium on N-methyl-D-aspartate receptors there by reducing excitatory discharge of neurons. In deficiency of zinc, these receptors get stimulated which may produce epileptiform discharges in children with fever[5].

According to Ganesh et al, zinc levels in febrile seizures children were lesser than febrile children. This indicates that zinc deficiency may be a important factor in febrile seizures pathogenesis[5]. Zinc also activates pyridoxal kinase, which in turn helps in the pyrioxal phosphate synthesis from

pyridoxal. Pyridoxal phosphate inturn activates glutamic acid decarboxylase which involved in synthesis of GABA. Post synaptic receptors in interaction with zinc assists in GABA action. Hence hypozincemia leads to decrease in GABA level which leads to development of seizures. According to Ehsanipour et al, zinc levels will be low in febrile seizures and during infection. But zinc levels were significantly low in patients with febrile seizures[53].

Diagnosis:

Deficiency of zinc can be estimated by measurement of zinc levels in serum by various methods. But atomic absorption spectrophotometer is most accurate method to estimate zinc levels. Confirmation of zinc deficiency can be done by zinc challenge are most accurate method[43].

Table 1: zinc levels

Zinc level in serum (µg/dL)	**Condition**
300 – 700	Acute zinc toxicity
160-299	Due to increased intake
84-159	Normal
60-83	Deficiency, fluctuations in zinc levels
< 59	Deficiency
<30	Definite deficiency

There may be variations in serum zinc levels depending on time of drawing blood and drugs etc[43].

Figure 19:

Condition	Change
Fasting	Increase
Food ingestion	Decrease (2–3 hours later)
Stress	Increase
Ingestion of marine products	Increase (oyster, etc.)
Neonates and infants	Decrease
Pregnancy	Decrease (gradually)
Drugs	
Glucocorticoids	Decrease
Thiazides	Increase
Loop diuretics	Increase
Disulfirams	Increase
Clofibrates	Decrease
Oral contraceptive pills	Decrease

Toxicity of zinc:

Excessive intake of zinc ranging from 150mg/day to 1-2 gm/day may cause toxicity of zinc. Toxicity will cause various deleterious effects on human body. Toxicity of zinc can be acute, sub chronic or sub chronic[36].

Table 2: Acute toxicity of zinc[36]

Toxicity	Effects
Inhalation of industrial fumes	Metal fume fever (chills,fever, chest pain, gastro enteritis, cough)
Dermal	Corrosive effects like blistering, ulcers, scar formation, contact dermatitis (rarely)
Ocular (zinc salts)	Corneal ulceration, hyperemia, hemorrhages, discrete grey spots on the lens
Gastro intestinal toxicity	Erosive esophagitis, pharyngitis, gastritis. Complications like G.I haemorrhage and pancreatitis.
Cardio vascular	Hypertension, premature atrial beats, shock
Hemotological	Microcytic anemia, leucopenia
Pulmonary	Broncho and laryngospasm leads to dysphonia, stridor and wheezing
Hepatic	Cholestatic liver disease to biliary cirrhosis, transiently elevated liver enzymes
Renal	Mild albuminuria, microscopic hematuria
Neurological	Lethargy, depression, coma, staggering
Oncological	Risk factor for cancer

Chronic toxicity of zinc[36]:

Zinc exposure for long periods leads to deficiency of copper. It also leads to hypoferremia and low haematocrit. It also has effects on various systems.

Treatment of deficiency of zinc:

Zinc will be supplemented for the treatment of zinc deficiency. The dose in children less than 6months will be 10mg/day and > 6months will be 20mg/day. This therapy should be continued for a period of 4 months. If therapy started with in 6months of deficiency, success rate of treatment is high[43].

Iron:

One of the important and widely prevalent nutritional problems throughout the world is deficiency of Iron. Along with developing countries, deficiency of Iron is widely prevalent in developed countries.

In developing countries, almost all children of pre-school age, and pregnant ladies had deficiency of Iron. In developed countries 30-40% of the preschool children and pregnant ladies had deficiency of Iron. In South Asia, anaemia is more prevalent than other parts of the world. In India, prevalence for pregnant and non-pregnant women was 88% and 74% respectively. Prevalence for preschool children in India was almost similar[54,55].

Figure 20: Sources of heme Iron[56]

Food (serving size)	Serving size	Iron content (mg)
Raisins	1 cup / 145 g	2.73
Apricots, dried	10 halves / 35 g	0.93
Bananas, fresh	1 banana / 118 g	0.31
Beans, chickpea (garbanzo)	1 cup, 164 g	4.74
Beans, kidney, canned	1 cup/ 256 g	3.25
Beans, green snap, canned	1 cup / 135 g	1.17
Beans, lentils, cooked	1 cup / 198 g	6.59
Beans, soy, mature cooked	1 cup, 172 g	8.84
Cereals, ready-to-eat, Cheerios	1 cup / 30 g	9.53
Cereals, ready-to-eat, Kellog's Product 19	1 cup, 30 g	18.09
Cornmeal, degermed, enriched yellow	1 cup / 138 g	5.96
Oats, cooked	1 cup / 234 g	2.11
Hummus	1 TBSP / 14 g	0.34
Wheat flour, white enriched, all purpose	1 cup / 125 g	5.80
Beans, black, cooked	1 cup / 172	3.61
Pumpkin, canned	1 cup / 245 g	3.41
Rice, white, enriched, long grain	1 cup / 185 g	7.97
Wheat flour, whole grain	1 cup / 120 g	4.66
Tofu, firm, Nigari	¼ block, 81 g	1.30
Tomato paste, canned	1 cup / 262 g	7.81
Greens, mustard, boiled and drained	1 cup / 140 g	.98
Broccoli, boiled and drained	1 cup / 156 g	1.05
Brussel sprouts, boiled and drained	1 cup / 156 g	1.87

Figure 21: Sources of non-haem iron

Food (serving size)	Serving size	Iron content (mg)
Beef, chuck, blade roast, trimmed to 1/8 inch fat	3 oz / 84 g	2.62
Beef, ground, 85% lean, broiled	3 oz / 85 g	2.21
Chicken, dark meat, fried	3 oz / 84 g	1.24
Chicken, light meat, fried	3 oz / 84 g	0.96
Clams, mixed species, canned and drained	3 oz / 85 g	23.77
Crustaceans, shrimp, mixed species, canned	3 oz / 85.05 g	1.81
Fish, cod, Pacific	3 oz / 85 g	0.28
Fish, haddock	3 oz / 85 g	1.15
Fish, pollock	3 oz / 85 g	0.24
Fish, salmon, canned, with bones and liquid	3 oz / 85 g	0.71
Fish, salmon, sockeye	3 oz / 85 g	0.47
Fish, tuna, fresh, yellowfin	3 oz / 85 g	0.80
Fish, tuna, light, canned in water and drained	3 oz / 85 g	1.30
Oysters, wild Eastern, raw	6 medium / 84 g	5.59
Pork, cured ham, lean, roasted	3 oz / 85 g	0.80
Turkey, dark meat, roasted	3 oz / 84 g	1.96
Turkey, light meat, roasted	3 oz / 84 g	1.13
Turkey, ground	1 patty / 82 g	1.58

Figure 22: Vitamin C sources to increase Iron absorption

TABLE 4 Selected Good Vitamin C Sources to Increase Iron Absorption

Fruits	Vegetables
Citrus fruits (eg, orange, tangerine, grapefruit)	Green, red, and yellow peppers
Pineapples	Broccoli
Fruit juices enriched with vitamin C	Tomatoes
Strawberries	Cabbages
Cantaloupe	Potatoes
Kiwifruit	Leafy green vegetables
Raspberries	Cauliflower

Figure 23 :Prevalence of anaemia based on concentration of haemoglobin[54]:

Percentage of total affected population in:	Industrialized countries	Non-industrialized countries
Children *(0-4 years)*	20.1	39.0
Children *(5-14 years)*	5.9	48.1
Pregnant women	22.7	52.0
All women *(15-59 years)*	10.3	42.3
Men *(15-59 years)*	4.3	30.0
Elderly *(+60 years)*	12.0	45.2

Epidemiology[54,56]**:**

Prevalence depends on various host factors. They are 1) age, 2) sex, 3) environmental, 4) physiological, 5) pathological, and 6) socio economic status. These factors influence dietary intake which in turn leads to deficiency.

1) Age: (ida assessment prevention control)

   In term infants, adequate stores of iron in liver and haematpoietic tissues are present. low levels of iron are present in breast milk ,so we have to compliment the food which can absorb more iron.

   Figure 24:

recommended iron intakes by age and gender group

Groups	Age (*years*)	Mean body weight (*kg*)	Required iron intake for growth (*mg/day*)	Median iron losses (*mg/day*)	
				Basal	Menstrual
Children	0.5-1	9.0	0.55	0.17	
	1-3	13.3	0.27	0.19	
	4-6	19.2	0.23	0.27	
	7-10	28.1	0.32	0.39	

2) Sex:After attaining menarche adolescents do not take sufficient amount of iron during their menstrual cycles. So, peak incidence is seen in adolescent females.

3) Environmental:

Folic acid, vitamins A, B 12, C and copper etc. may also necessary for haematopoiesis along with iron may also be deficient. Any trauma or any chronic systemic illness in the childhood may cause iron deficiency.

4&5)Physiological &Pathological:

6) Socio-economic status: Common in below poverty line population.

<u>Iron metabolism</u>:

Figure 25: Below diagram showing absorption and metabolism of Iron in the gut.

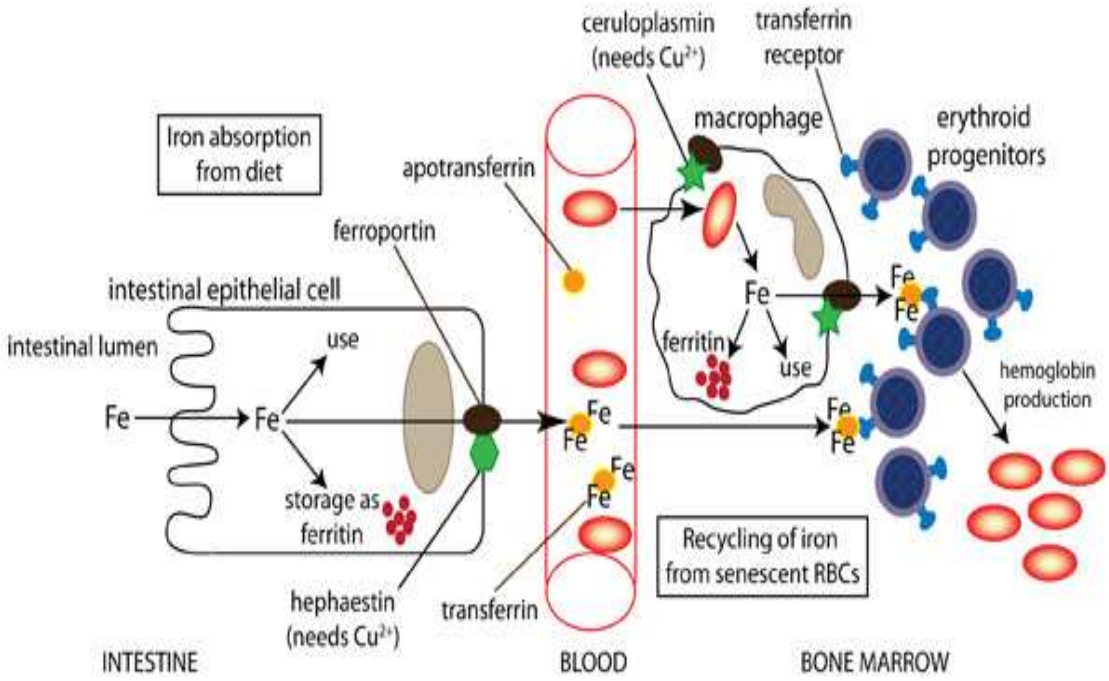
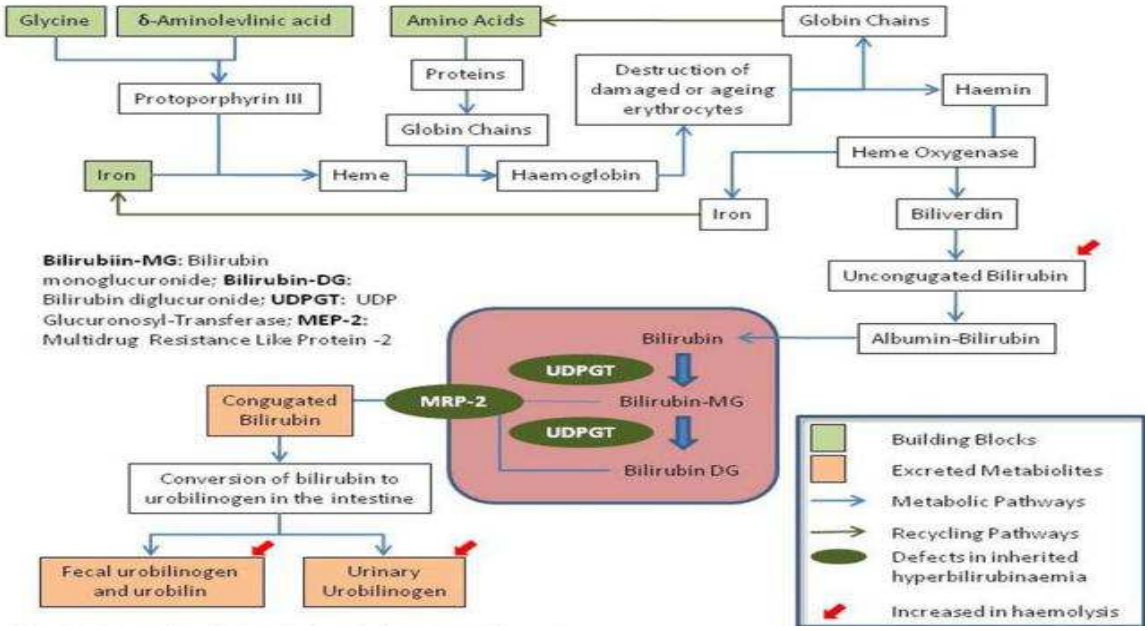


Figure Hemoglobin production and catabolism:



Role of Iron deficiency in febrile seizures:

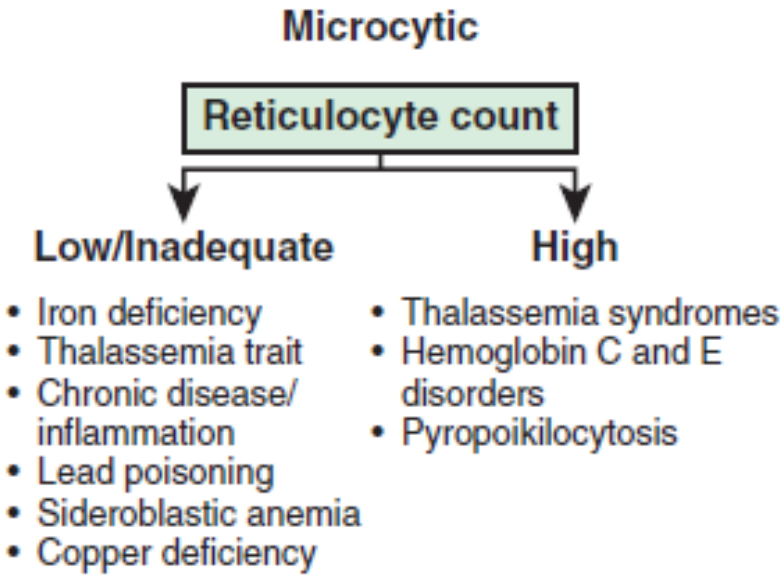
Iron is an important element for metabolism in the brain. It also helps in neuro transmitter metabolism. Deficiency of iron acts as an important factor in development of febrile seizures. Iron deficiency is one of most common nutritional problem around the world. Kumari et al. conducted a case control study involving large sample size. They reported that deficiency of iron was seen in majority of the patients. They concluded that, deficiency of iron is one of the important factors in development of febrile convulsions[6]. According to a study conducted in Kenya, deficiency of iron is not a risk factor for other acute convulsions. But it acts as important factor in febrile convulsions[57].

Signs&Symptoms[54,56,58]**:**

Symptoms of iron deficiency can be very mild at first,and completely un noticed.

- General fatigue
- Weakness
- Pale skin
- Shortness of breath
- Dizziness
- Pica
- Tingling/crawling feeling in the legs
- Swelling /soreness in the tongue
- Cold hands & feet
- Fast/irregular heart beat
- Brittle nails
- Headaches
- Hair loss
- Twitches
- Restless legs syndrome

Approach to iron deficiency anaemia[58]: (Figure 27)



How to differentiate from other microcytic anaemia? (Figure 28)

LABORATORY STUDIES DIFFERENTIATING THE MOST COMMON MICROCYTIC ANEMIAS

STUDY	IRON DEFICIENCY ANEMIA	α OR β THALASSEMIA	ANEMIA OF CHRONIC DISEASE
Hemoglobin	Decreased	Decreased	Decreased
MCV	Decreased	Decreased	Normal-decreased
RDW	Increased	Normal	Normal-increased
RBC	Decreased	Normal-increased	Normal-decreased
Serum ferritin	Decreased	Normal	Increased
Total Fe binding capacity	Increased	Normal	Decreased
Transferrin saturation	Decreased	Normal	Decreased
FEP	Increased	Normal	Increased
Transferrin receptor	Increased	Normal	Increased
Reticulocyte hemoglobin concentration	Decreased	Normal	Normal-decreased

Indicators of Iron deficiency Anaemia[58]: (Figure 29):

INDICATOR	SELECTED CUTOFF VALUES TO DEFINE IRON DEFICIENCY	COMMENTS
Hemoglobin (g/L)	6 mo-5 yr <110	When used alone, it has low specificity and sensitivity
	6-11 yr <115	
	Nonpregnant women <120	
	Pregnant women <110	
Mean corpuscular volume (MCV) (μm^3)	Children older than 11 yr and adults <82	A reliable, but late indicator of iron deficiency Low values can also be due to thalassemia
Reticulocyte hemoglobin content (CHr) (pg)	In infants and young children <27.5 In adults ≤28.0	A sensitive indicator that falls within days of onset of iron-deficient erythropoiesis False normal values can occur when MCV is increased and in thalassemia Wider use is limited because it can only be measured on a few analyzer models
Erythrocyte zinc protoporphyrin (ZPP) (μmol/mol heme)	≤5 yr >70 Children >5 yr >80 Children >5 yr on washed red cells >40	It can be measured directly on a drop of blood with a portable hematofluorometer A useful screening test in field surveys, particularly in children, in whom uncomplicated iron deficiency is the primary cause of anemia Red cells should be washed before measurement because circulating factors, including serum bilirubin, can spuriously increase values Lead poisoning can increase values, particularly in urban and industrial settings
Transferrin saturation	<16%	It is inexpensive, but its use is limited by diurnal variation in serum iron and by many clinical disorders that affect transferrin concentrations
Serum ferritin (SF) (μg/L)	≤5 yr <12 Children >5 yr <15 In all age groups in the presence of infection <30	It is probably the most useful laboratory measure of iron status; a low value of SF is diagnostic of iron-deficiency anemia in a patient with anemia In healthy persons, SF is directly proportional to iron stores: 1 μg/L SF corresponds to 8-10 mg body iron or 120 μg storage iron per kg body weight As an acute-phase protein, SF increases independent of iron status by acute or chronic inflammation; it is also unreliable in patients with malignancy, hyperthyroidism, liver disease, or heavy alcohol intake
Serum transferrin receptor (sTfR)	Cutoff varies with assay and with patient's age and ethnic origin	Main determinants are the erythroid mass in the bone marrow and iron status; thus, sTfR is increased by enhanced erythropoiesis and iron deficiency sTfR is not substantially affected by the acute-phase response, but it might be affected by malaria, age, and ethnicity Its application is limited by high cost of commercial assays and lack of an international standard
sTfR: SF ratio		This ratio is a quantitative estimate of total body iron; the logarithm of this ratio is directly proportional to the amount of stored iron in iron-replete patients and the tissue iron deficit in iron deficiency In elderly people, this ratio might be more sensitive than other laboratory tests for iron deficiency This ratio cannot be used in patients with inflammation because SF might be high independent of iron stores This ratio is assay specific Although it is only validated for adults, this ratio has been used in children

Differential diagnosis of iron deficiency:

1) Thalassemia
2) Haemoglobin C and E disorders
3) Anaemia of chronic disease
4) Lead poisoning
5) Sickle cell anemia

Management of Iron deficiency[56,58]:

Deficiency of Iron can be treated by supplementation of elemental iron.

Iron can be given in 2 types of preparations

1. Oral iron preparation
2. Parenteral iron preparation

Oral iron therapy:

Dose should be calculated for elemental iron. 3-6mg/kg/day in 3 divided doses can be given. Common forms used are ferrous sulphate which contains elemental iron of 20%. It should be given in meals along with juice to increase compliance.

Side effects of oral Iron preparations:

Gastro intestinal disturbances such as epigastric pain, irregular bowel habits etc. Proton pump inhibitors may also decrease the absorption of iron

Response to iron therapy: (Figure 30)

TIME AFTER IRON ADMINISTRATION	RESPONSE
12-24 hr	Replacement of intracellular iron enzymes; subjective improvement; decreased irritability; increased appetite
36-48 hr	Initial bone marrow response; erythroid hyperplasia
48-72 hr	Reticulocytosis, peaking at 5-7 days
4-30 days	Increase in hemoglobin level
1-3 mo	Repletion of stores

Parenteral Iron therapy[54]:

parenteral therapy is indicated in the presence of malabsorption or who are not responsive to Iron therapy. Various parenteral Iron preparations are available.

RESULTS

Study population included 100 patients divided into two groups. Two groups included 50 cases (febrile seizures) and 50 controls (febrile children without seizures) that came to out-patient department or admitted as in-patient. Table 3 describes about characteristics of both groups. Age, Nutritional status were matched in both groups.

Table 3: Characteristics of patients in both groups

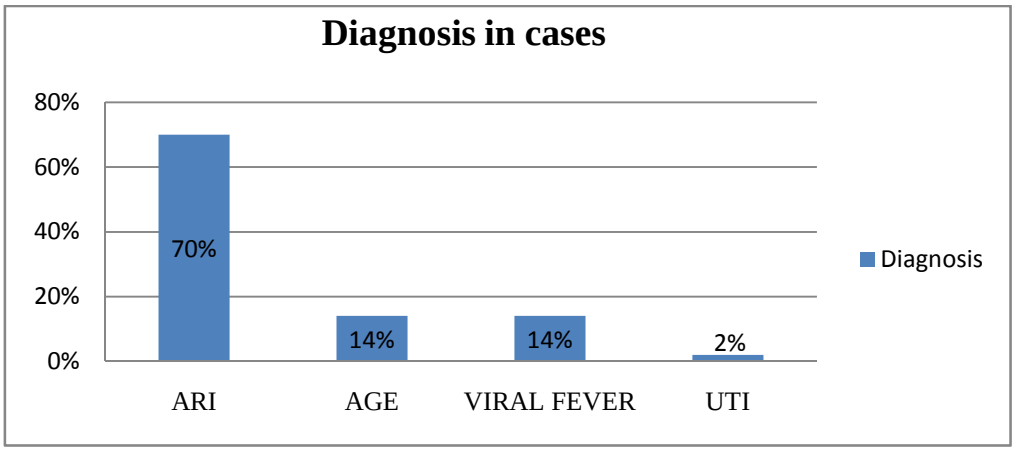
	febrile seizures (n=50)	Fever without seizures (n=50)	p-value
Sex (male:female) Male female	1.17:1 27 23	 33 17	>0.05
Mean age(in months)	22.62 ± 12.45	23.14 ± 15.58	>0.05
Nutritional status Normal Grade I malnutrition Grade II malnutrition Grade III malnutrition Grade IV malnutrition	 40 10 Nil Nil Nil	 40 10 Nil Nil Nil	1.00
Family history of febrile seizures	11	**0**	0.002
Mean temperature	101.61 ± 1.31	101.17 ± 0.86	>0.05
Socio economic status Upper Upper middle Middle Upper lower Lower	 1 9 5 11 24	 1 6 7 15 21	0.782
Duration of seizure <5mins 5-10mins 10-15mins	 **40** **10** **0**		

In the study population, 70% of the cases (35/50) had acute respiratory infection, 14% of the cases (7/50) each had AGE and Viral fever. 2% of the cases had UTI (1/50). 54% of the controls (27/50) had ARI. 24% of the controls (12/50) had viral fever. 20% of the controls (10/50) had AGE. 2% of the controls had UTI (1/50) (**Table 4, Figure 31 &32**).

Table 4: Diagnosis in cases and controls

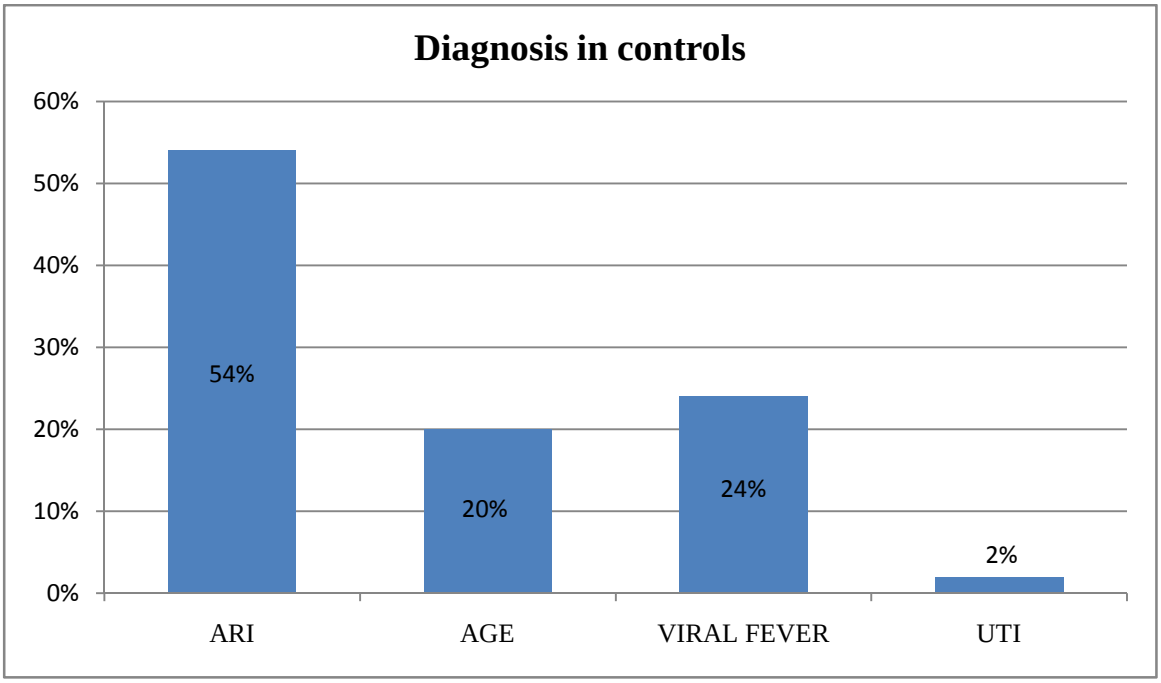
	Cases (n=50)		Controls (n=50)	
Diagnosis	Number of patients	Percentage	Number of patients	Percentage
ARI	35	70%	27	54%
AGE	7	14%	10	20%
VIRAL FEVER	7	14%	12	24%
UTI	1	2%	1	2%
TOTAL	50	100%	50	100%

Figure 31:



ARI is the predominant diagnosis followed by acute gastroenteritis (14%), viral fever (14%) and UTI (2%) respectively. (**Table 4and figure 31**).

Figure 32:

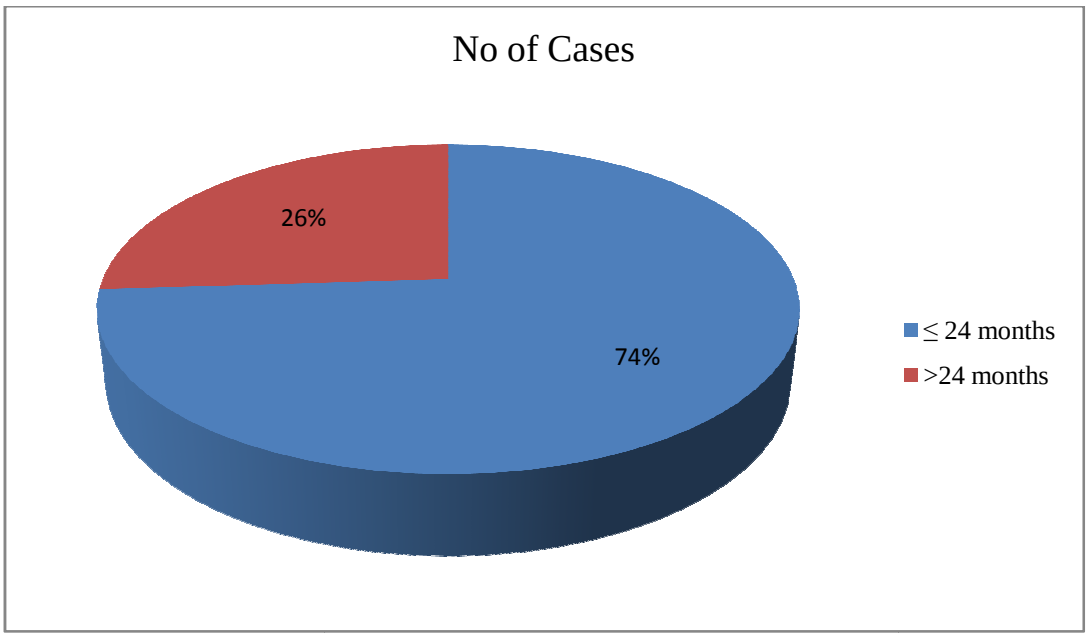


ARI is the predominant diagnosis in controls (54%) followed by viral fever (24%), AGE (20%) and UTI (2%) respectively. (**Table 4 and figure 32**)

TABLE 5: Age distribution of cases and controls

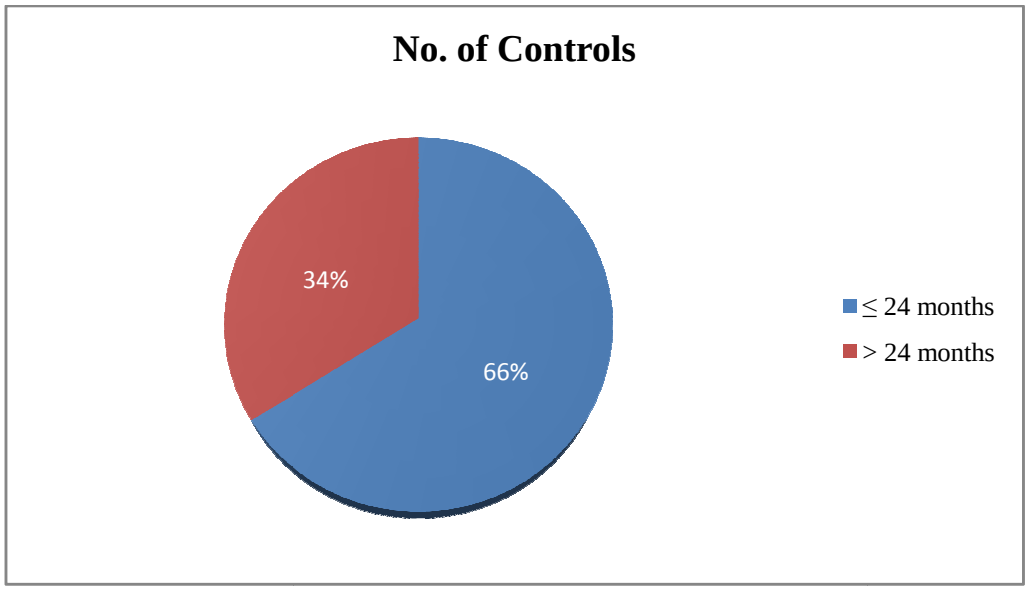
	Cases (n=50)		Controls (n=50)	
AGE GROUP(months)	NO. OF PATIENTS	PERCENTAGE	NO. OF PATIENTS	PERCENTAGE
≤ 24 MONTHS	37	74%	33	66%
>24 MONTHS	13	26%	17	34%
TOTAL	50	100%	50	100%

Figure 33:



74% of the cases (37) belong to ≤24months age group and 26% of the cases (13) belong to >24months age group. (**Table 5 and figure 33**)

Figure 34:

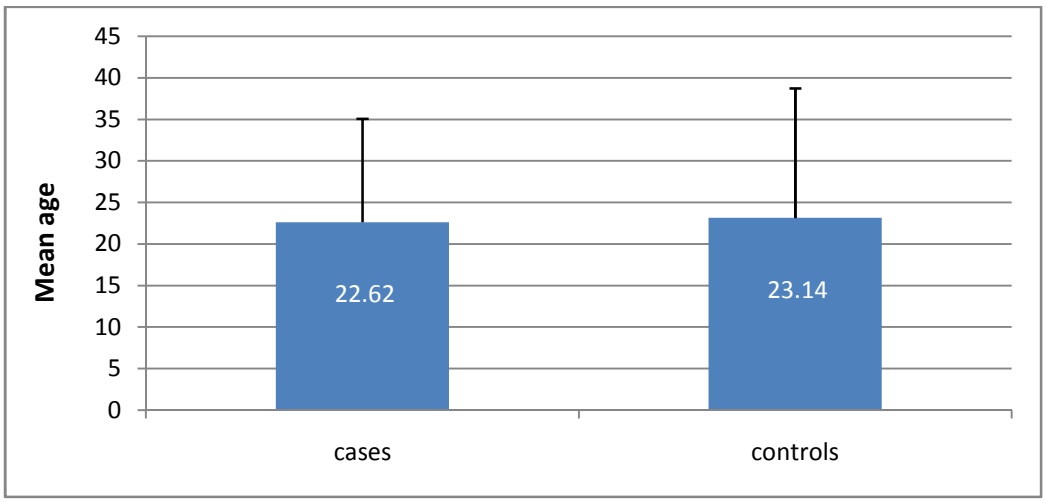


66% of the controls (33) belong to ≤24months age group and 34% of the controls (17) belong to >24months age group. (Table 5 and figure 34)

TABLE 6:

	CASES (n=50)	CONTROLS (n=50)
MEAN	22.62	23.14
STANDARD DEVIATION	12.45	15.58

Figure 35:

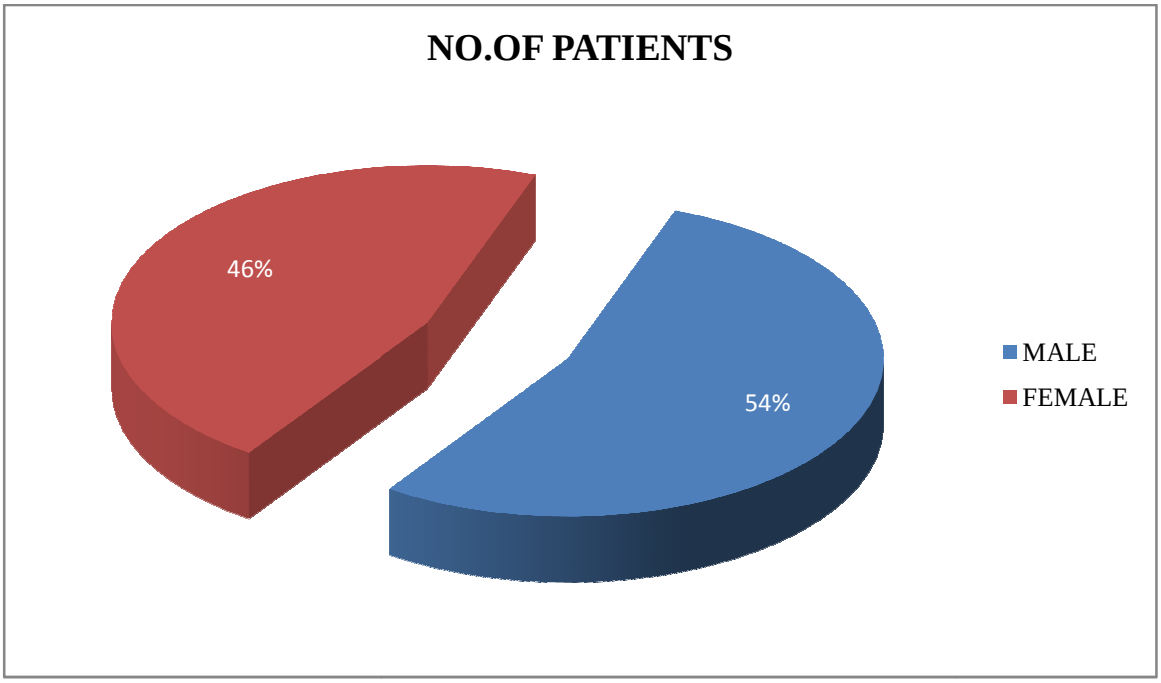


Mean age in cases and controls were 22.62 ± 12.45 and 23.14 ± 15.58 months respectively. The difference was not statistically significant (p-value is >0.05). (**Table 6 and figure 35**).

Table 7: Gender distribution of cases and controls

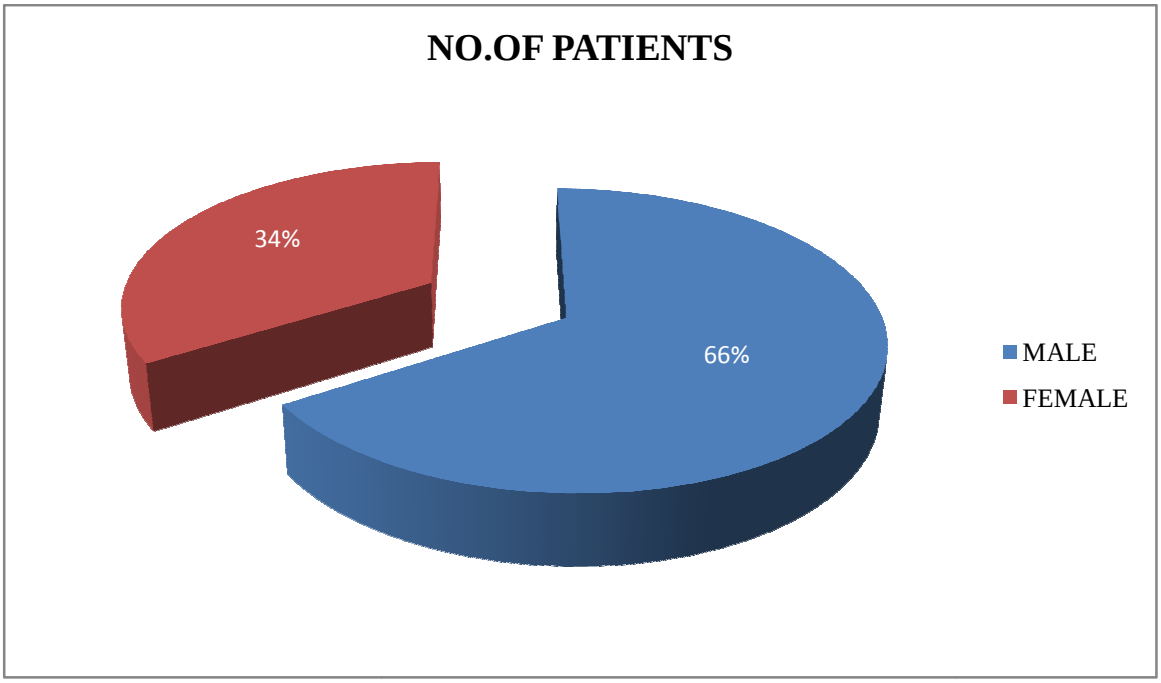
	CASES (n=50)		CONTROLS (n=50)	
GENDER	NO.OF PATIENTS	PERCENTAGE	NO.OF PATIENTS	PERCENTAGE
MALE	27	54%	33	66%
FEMALE	23	46%	17	34%
TOTAL	50	100%	50	100%

Figure 36: Gender distribution of cases



54% of the cases were male (27) and 46% of the cases were female (23). Male: female ratio was 1.17:1. (**Table 7 and figure 36**)

Figure 37: Gender distribution of controls

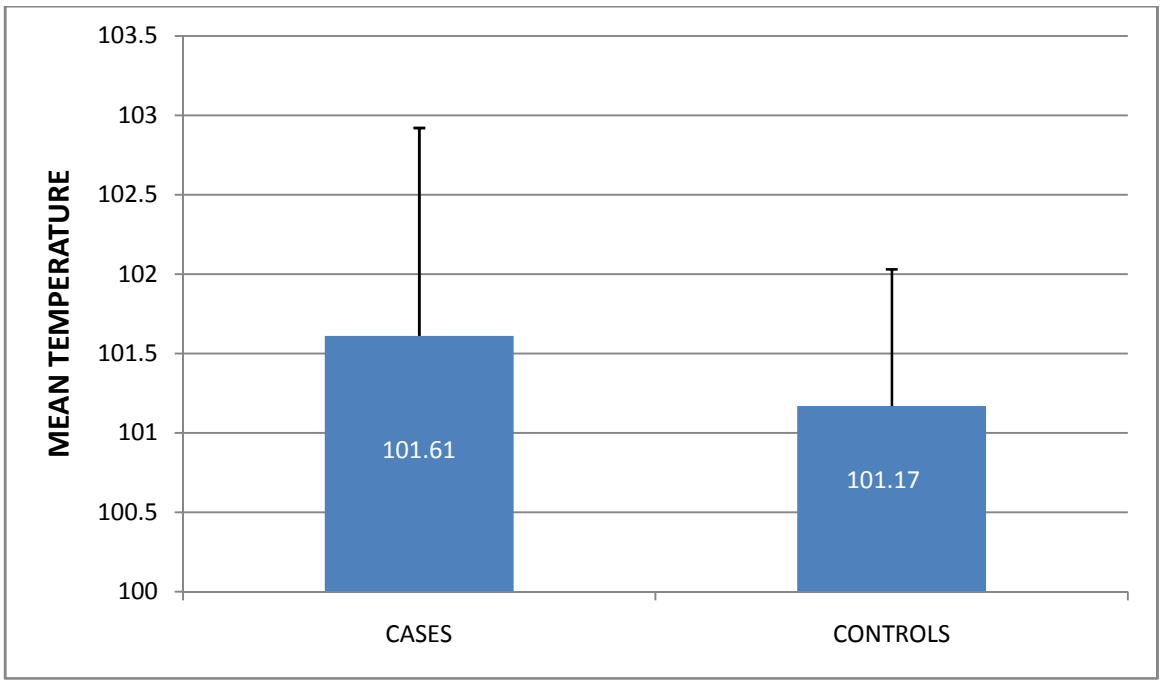


66% of the controls (33) were male and 34% of the controls (17) were female. There is no statistical difference between two groups (p- value >0.05). (Table 7 and figure 37)

TABLE 8: Temperature in cases and controls

	CASES (n=50)	CONTROLS (n=50)
MEAN	101.61	101.17
STANDARD DEVIATION	1.31	0.86

Figure 38:



Mean temperature in cases and controls were 101.61 ± 1.31°F and 101.17 ± 0.86°F respectively. The difference between two groups was not statistically significant (p-value >0.05). (**Table 8 and figure 38**)

Table 9: Family History of Febrile Seizure

	CASES (n=50)		CONTROLS (n=50)	
FAMILY H/O FEBRILE SEIZURE	NO.OF PATIENTS	PERCENTAGE	NO. OF PATIENTS	PERCENTAGE
PRESENT	11	22%	0	0%
ABSENT	39	78%	50	100%
TOTAL	50	100%	50	100%

Figure 39: family history of febrile seizures

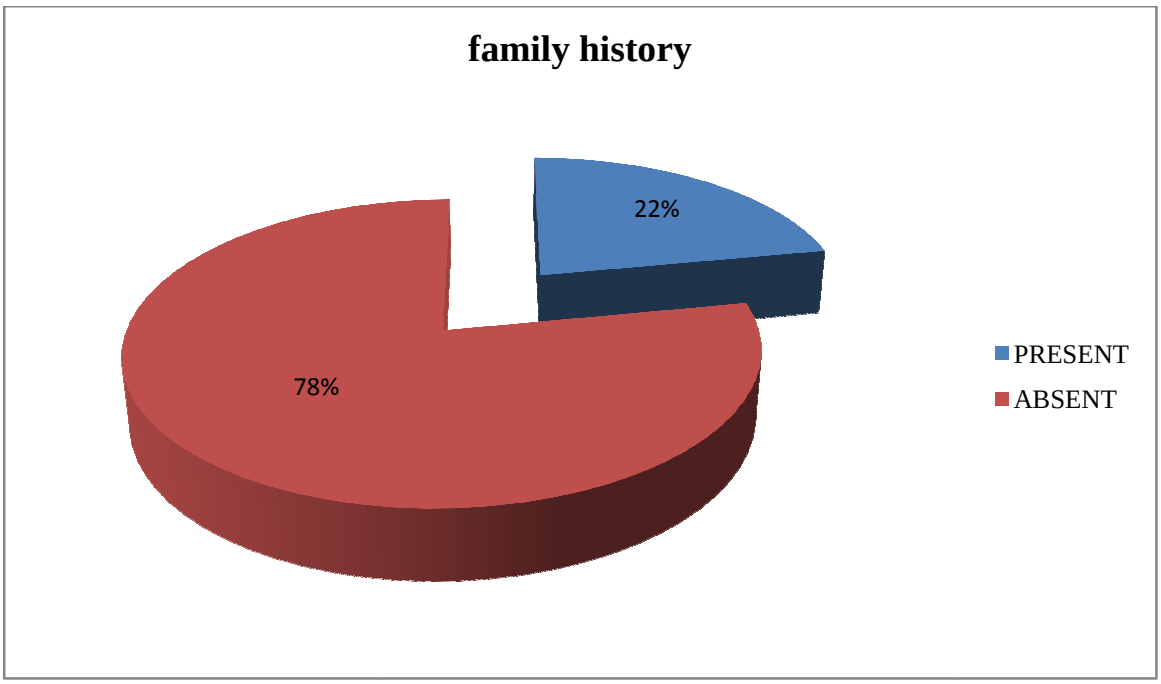


Table 10:

ACTUAL VALUES			
FAMILY H/O FEBRILE SEIZURES	CASE	CONTROL	TOTAL PATIENTS
PRESENT	11	0	11
ABSENT	39	50	89
TOTAL	50	50	100

Table 11:

EXPECTED VALUES			
FAMILY H/O FEBRILE SEIZURES	CASE	CONTROL	TOTAL PATIENTS
PRESENT	5.5	5.5	11
ABSENT	44.5	44.5	89
TOTAL	50	50	100

Table 12:

Chi Test p-value	0.002
Chosen significance value	0.05

22% of cases (11/50) had family history of febrile seizures. In controls there was no family history of febrile seizures. The difference between two groups was statistically significant (p-value 0.002). (Table 9,10,11,12 & figure 39)

Table 13: Socio economic status

	Cases (n=50)		Controls (n=50)	
SOCIO ECONOMIC STATUS	No.of patients	Percentage	No. of patients	Percentage
UPPER	1	2%	1	2%
UPPER MIDDLE	9	18%	6	12%
MIDDLE/LOWER MIDDLE	5	10%	7	14%
LOWER/UPPER LOWER	11	22%	15	30%
LOWER	24	48%	21	42%
TOTAL	50	100%	50	100%

Figure 40:

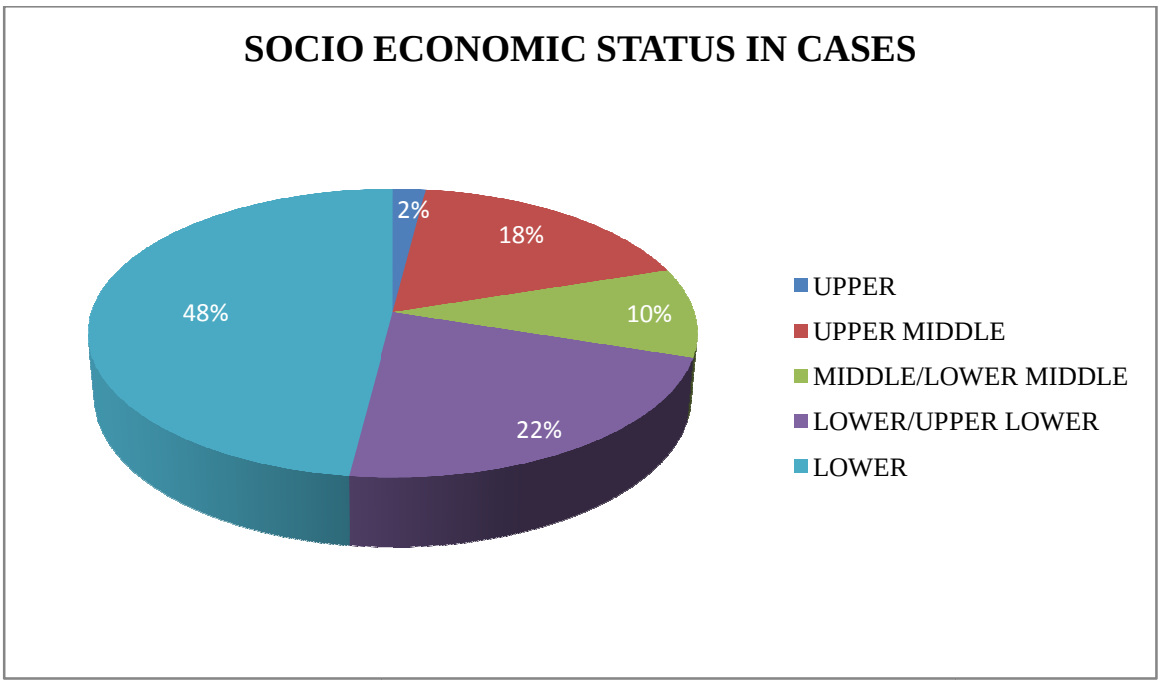


Figure 41:

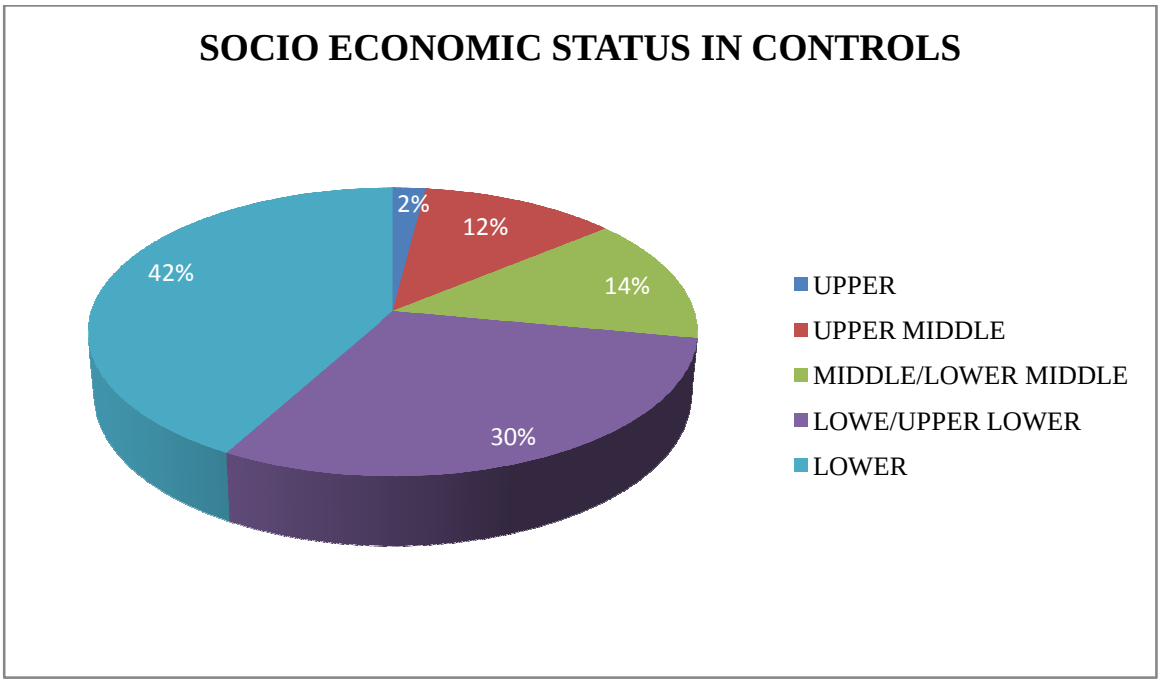


Table 14:

ACTUAL VALUES			
SOCIOECONOMIC STATUS	CASE	CONTROL	TOTAL PATIENTS
UPPER	1	1	2
UPPER MIDDLE	9	6	15
MIDDLE/LOWER MIDDLE	5	7	12
LOWER/UPPER LOWER	11	15	26
LOWER	24	21	45
TOTAL	50	50	100

Table 15:

EXPECTED VALUES			
SOCIOECONOMIC STATUS	CASE	CONTROL	TOTAL PATIENTS
UPPER	1	1	2
UPPER MIDDLE	7.5	7.5	15
MIDDLE/LOWER MIDDLE	6	6	12
LOWER/UPPER LOWER	13	13	26
LOWER	22.5	22.5	45
TOTAL	50	50	100

Table 16:

Chi Test p-value	0.782
Chosen significance value	0.05

Lower socio economic status group was the predominant group (48%) in cases followed by upper lower (22%), upper middle (18%), lower middle (10%) and upper (2%) respectively. In controls, Lower socio economic group was the predominant group (42%) followed by upper lower (30%), lower middle (14%), upper middle (12%) and upper (2%) respectively. The difference between both groups was not significant (p-value 0.782). (Table 13,14,15,16 & figure 40, 41)

Table 17: Nutritional status

	Cases (n=50)		Controls (n=50)	
NUTRITIONAL STATUS	No.of patients	Percentage	No. of patients	Percentage
NORMAL	40	80%	40	80%
GRADE (I)	10	20%	10	20%
GRADE (II)	0	0%	0	0%
GRADE (III)	0	0%	0	0%
GRADE (IV)	0	0%	0	0%
TOTAL	50	100%	50	100%

Figure 42:

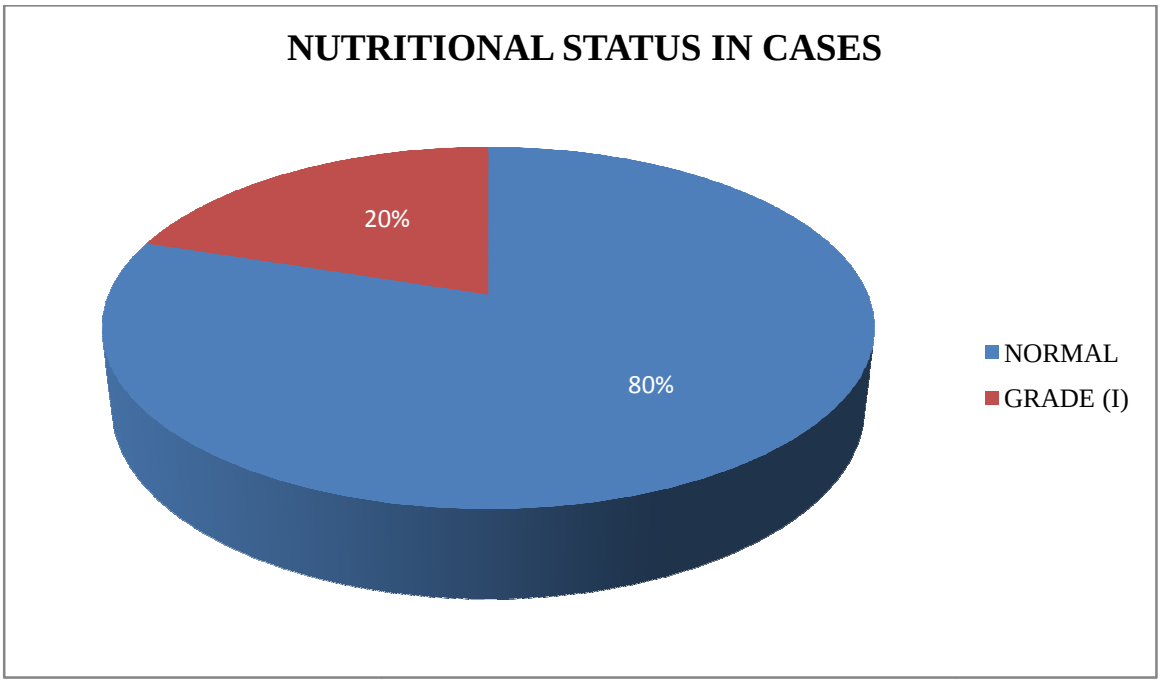
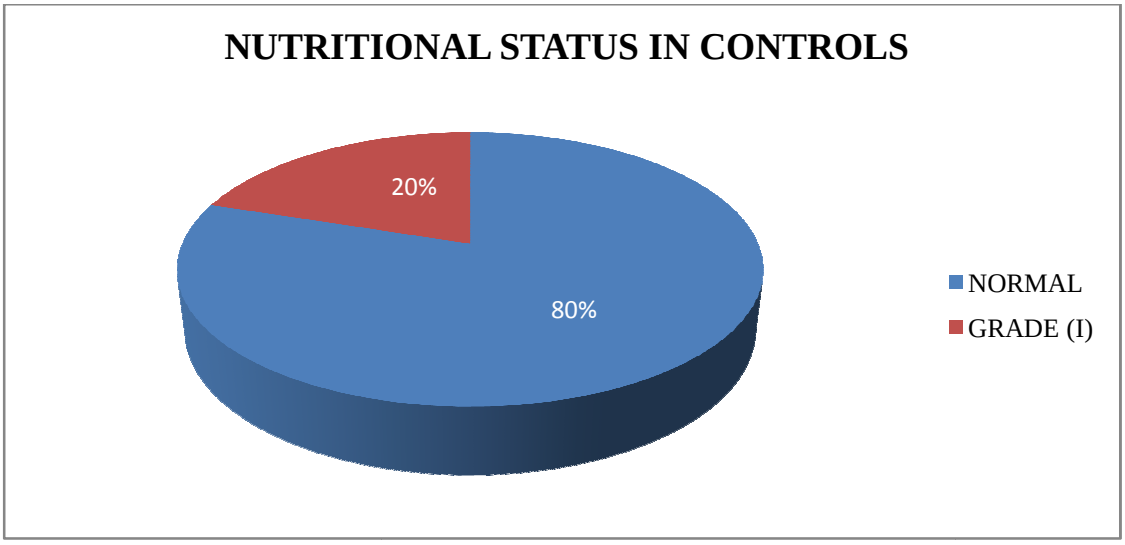


Figure 43:

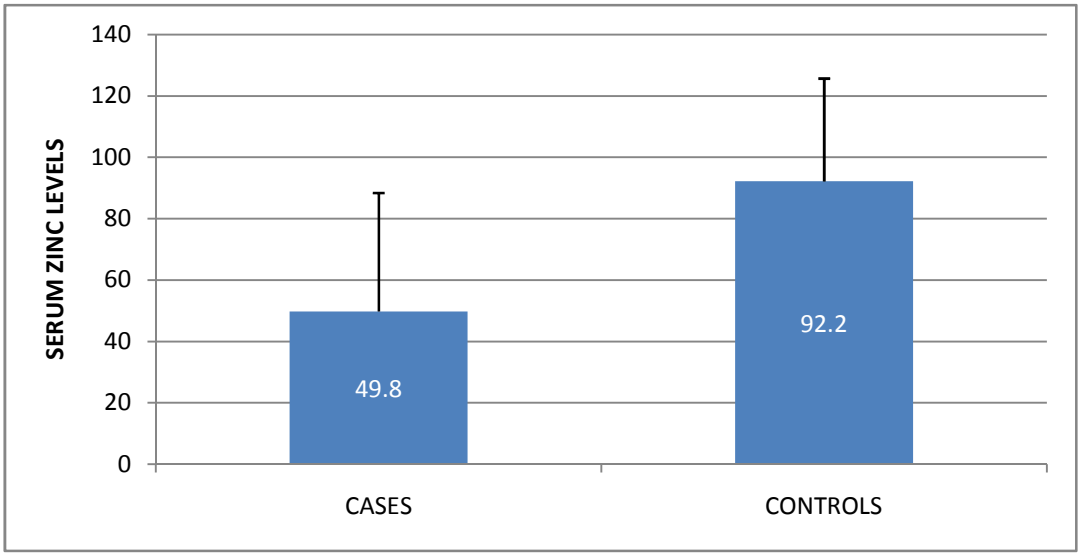


80% of the cases (40) had normal nutritional status and 20% of the cases (10) had grade-I malnutrition. In controls, 80% (40) had normal nutritional status and 20% (10) had grade-1 malnutrition. The difference between two groups is not statistically significant (p-value 1.000). (Table 17, figure 42&43)

Table 18: Mean values of all investigations in cases and controls

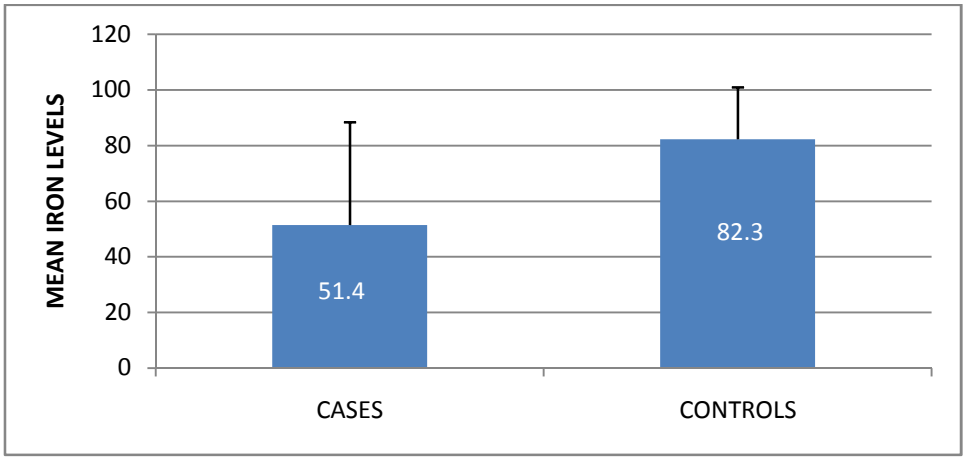
	Cases		Controls		
Investigations	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**p- value**
Sr. Iron	**51.4**	**37.0**	**82.3**	**18.6**	**<0.0001**
Haemoglobin	**10.6**	**0.9**	**11.6**	**0.8**	**<0.0001**
MCV	**68.7**	**6.3**	**76.5**	**6.5**	**<0.0001**
MCHC	**33.1**	**1.4**	**33.6**	**1.1**	**0.0629**
MCH	**22.8**	**2.4**	**26**	**2.6**	**<0.0001**
RDW	**16.1**	**1.6**	**14.3**	**1.5**	**<0.0001**
Sr. Zinc	**49.8**	**38.6**	**92.2**	**33.5**	**<0.0001**

Figure 44: Mean serum zinc levels in cases and controls



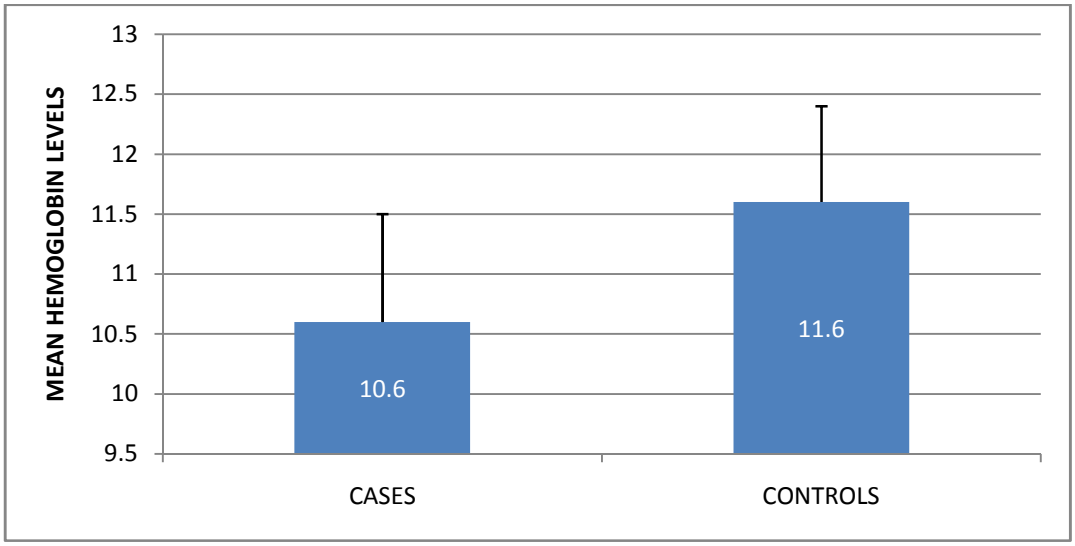
Mean serum Zinc levels in cases and controls were 49.8 ± 38.6 and 92.2 ±33.5 µg /dl respectively. Mean zinc levels were low in cases. With t = 5.8539 and with 95% confidence interval, p-value is < 0.0001. It was strongly significant. Lowest Zinc level was 14 µg /dL. Highest Zinc level was 126µg/dL. (**Table 18 & figure 44)**

Figure 45: Mean serum iron levels in cases and controls



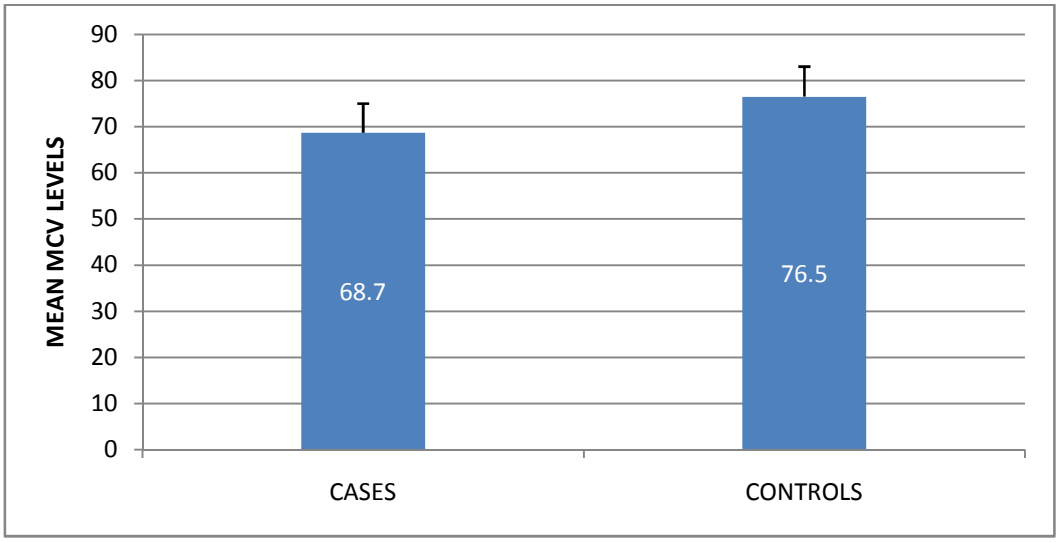
Mean serum iron levels in cases and controls were 51.4 ± 37 and 82.3 ± 18.6µg /dl.Mean serum iron levels were low in cases than controls. With t = 5.2719, and 95% confidence interval, p value is <0.0001. It was statistically significant. Lowest iron level was 13µg/dL and highest Iron level was 67.4 in cases. (**Table 18 & figure 45**)

Figure 46: Mean haemoglobin in cases and controls:



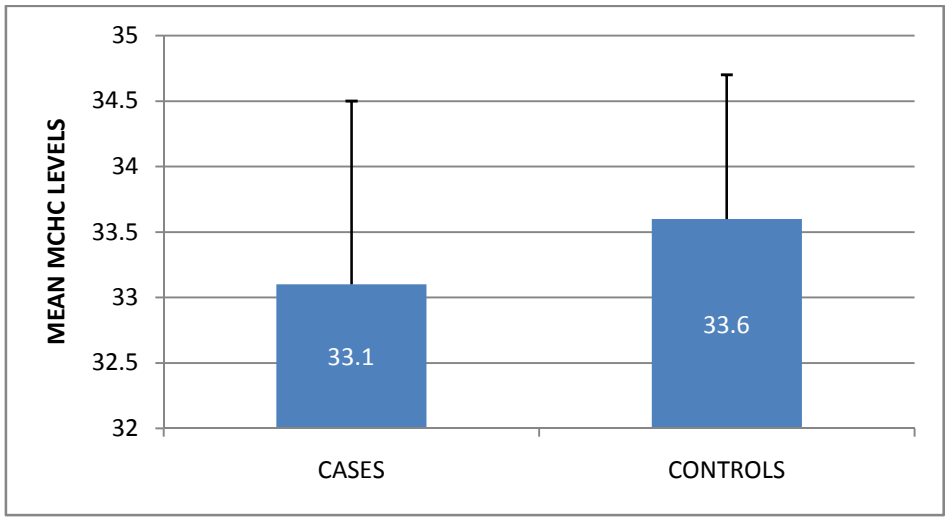
Mean Haemoglobin levels in cases and controls were 10.6 ± 0.9 and 11.6 ± 0.8 gm/dl respectively. With t = 5.74,and with 95% confidence interval, p- value is <0.0001, it was strongly significant. Lowest haemoglobin level was 8.6g/dL. Highest haemoglobin level was 12.2g/dL. (**Table 18& figure 46**)

Figure 47: Mean MCV levels in cases and controls



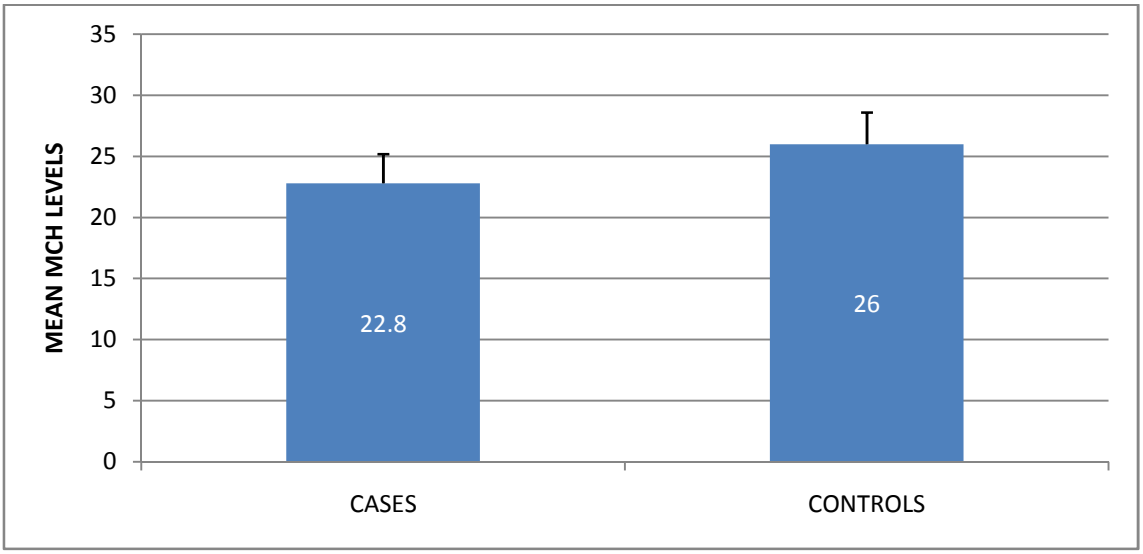
Mean MCV levels in cases and controls were 68.7 ± 6.3 and 76.5 ± 6.5 flrespectively. With t = 6.062, and with 95% confidence interval, p-value is <0.0001. It was strongly significant. Lowest MCV value was 52.8 and highest MCV value was 82.8 fl. (**Table 18& figure 47**)

Figure 48: Mean MCHC levels in cases and controls



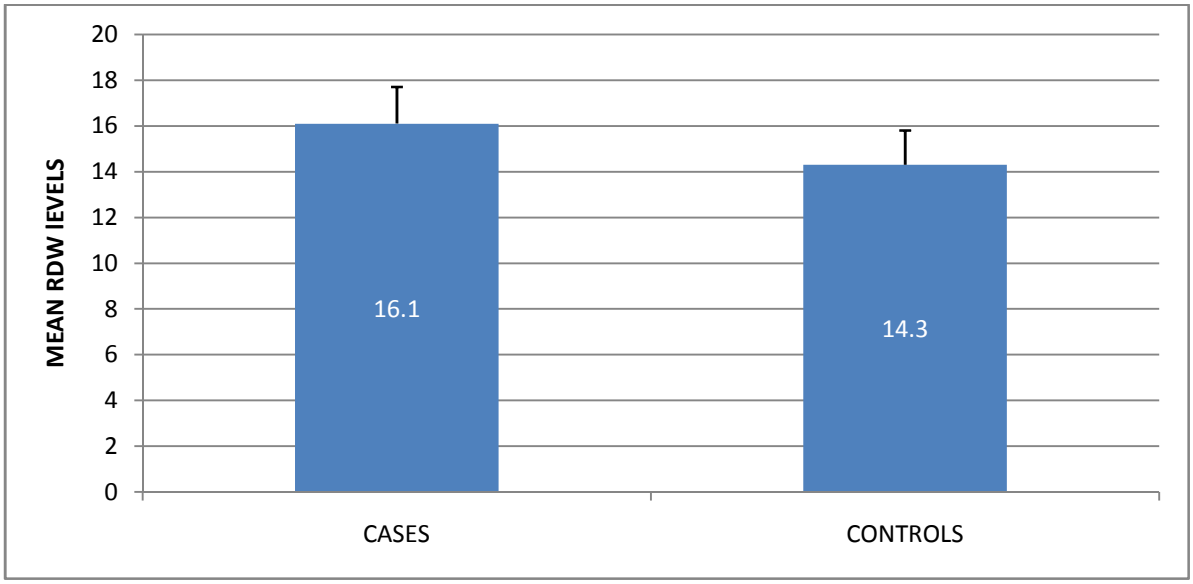
Mean MCHC levels in cases and controls were 33.1 ± 1.4 and 33.6 ± 1.1 % respectively. There was no significant difference between 2 groups (P-value is 0.0629). Highest MCHC value was 34.6 % and lowest MCHC value was 30.4 %. (**Table 18& figure 48**)

Figure 49: Mean MCH levels in cases and controls



Mean MCH levels in cases and controls were 22.8 ± 2.4 and 26 ± 2.6pg respectively. With t = 6.4332, and with 95% confidence interval, p-value is <0.0001. It was strongly significant. Lowest MCH value was 18.2pg and highest MCH value was 24.6pg. (**Table 18 & figure 49**).

Figure 50: Mean RDW levels in cases and controls



Mean RDW values in cases and controls were 16.1 ± 1.6 and 14.3 ± 1.5 % respectively. With t = 6.321, and with 95% confidence interval, p value is <0.0001. It was strongly significant. Highest RDW value was 20.8%. Lowest RDW value was 15.1%. (**Table 18& figure 50**)

Table 19: Mean values of all investigations in <24 months age group:

	Cases		Controls		
Investigations	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**p- value**
Sr. Iron	**47.09**	**33.3**	**80.6**	**21.07**	**<0.0001**
Haemoglobin	**10.4**	**0.7**	**11.5**	**0.8**	**<0.0001**
MCV	**68.11**	**5.5**	**74.5**	**6.7**	**<0.0001**
MCHC	**32.9**	**1.4**	**33.4**	**1.1**	**0.098**
MCH	**22.6**	**2.3**	**25.3**	**2.7**	**<0.0001**
RDW	**16.3**	**1.6**	**14.6**	**1.68**	**<0.0001**
Sr. Zinc	**49.8**	**38.6**	**92.2**	**33.5**	**<0.0001**

Table 20: Mean values of all investigations in >24 months age group

	Cases		Controls		
Investigations	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**p- value**
Sr. Iron	**63.5**	**45.05**	**85.3**	**12.57**	**0.067**
Haemoglobin	**11**	**1.32**	**11.8**	**0.58**	**0.032**
MCV	**70.2**	**8.2**	**80.2**	**4.2**	**<0.0001**
MCHC	**33.5**	**1.4**	**33.7**	**1.1**	**0.544**
MCH	**23.3**	**2.5**	**27.3**	**1.4**	**<0.0001**
RDW	**15.4**	**1.5**	**13.6**	**0.65**	**<0.0001**
Sr. Zinc	**49.6**	**42.1**	**99.7**	**35.7**	**<0.001**

Table 19 and 20 showed that mean levels of serum iron and other red cell indices were comparatively lower in <24 months age group than >24 months age group. In >24 months age group, mean values of haemoglobin, MCHC, iron were not statistically significant. In both <24 months and >24 months age group, mean serum zinc levels were significantly lower (p-value: <0.0001 and <0.001 respectively)

Table 21: Duration of seizure

Duration of seizure	No of patients	Percentage
<5mins	**40**	**80%**
5-10mins	**10**	**20%**
>10mins	**0**	**0%**

Figure 51:

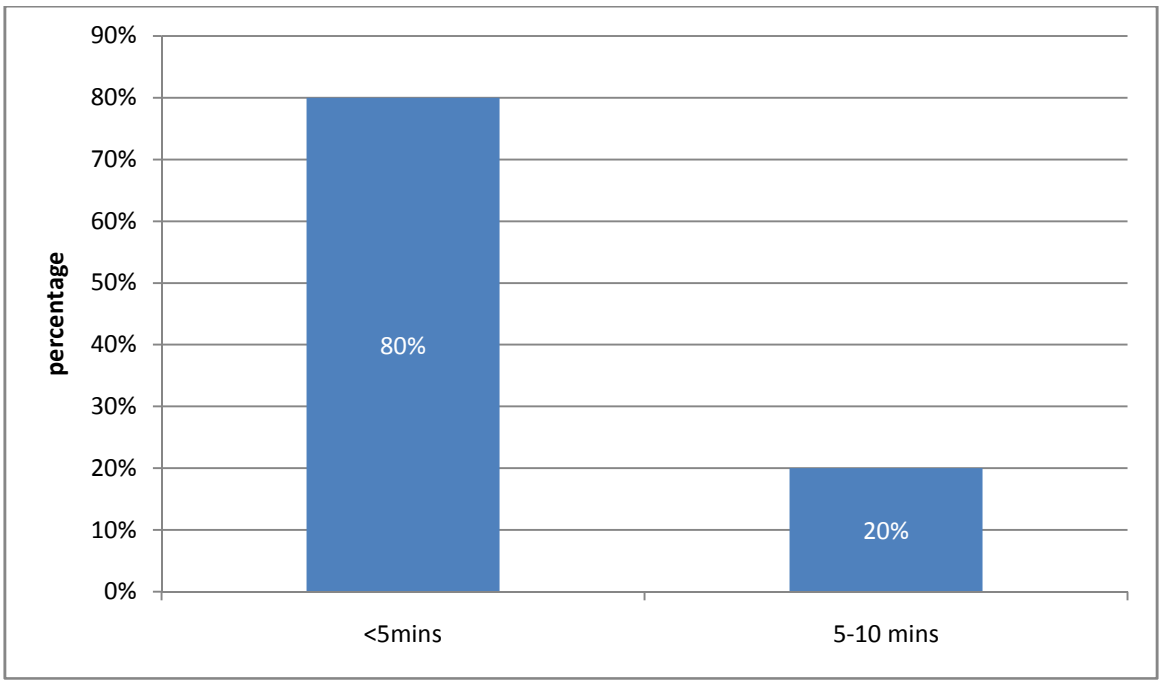
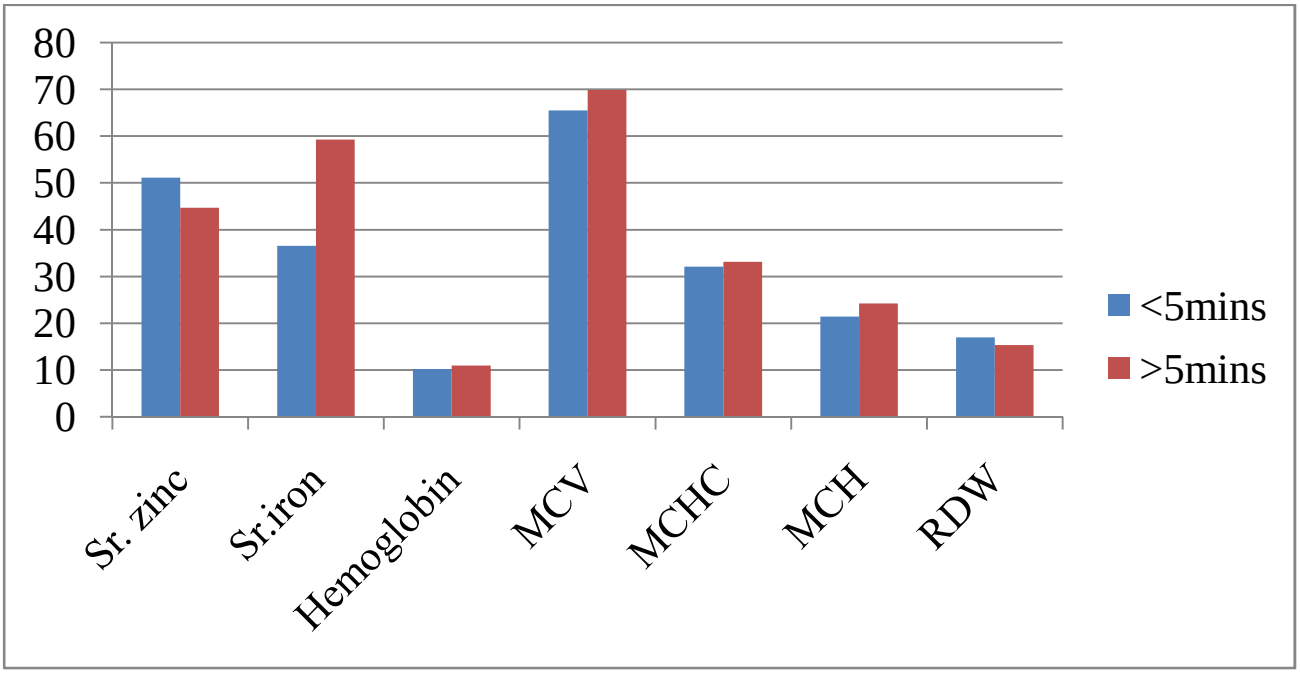


Table 21 and figure 51 showed that duration of seizure was < 5minutes in 80% of cases (40/50) and >5mins in 20% of the cases (10/50).

Figure 52: **Comparison of mean levels between <5minutes and 5-10 minutes group**



Mean zinc levels were comparatively lower in 5-10 minutes group when compared with <5minutes group. Mean iron levels and red cell indices levels were lower in <5minutes group when compared with 5-10 minutes group. There was no significant difference between two groups (p-value > 0.0001). (Figure 52)

Table 22: Odds ratios

		Cases	Controls	Total	
Only low Iron	Present %	6 60%	4 40%	10 100%	Odds ratio: 1.568 Relative risk: 1.227 p- value: 0.507
	Absent %	44 48.9%	46 51.1%	90 100%	
Total	Count %	50 50%	50 50%	100 100%	

		Cases	Controls	Total	
Only low zinc	Present %	4 36.4%	7 63.6%	11 100%	Odds ratio: 0.534 Relative risk: 0.704 p- value: 0.343
	Absent %	46 51.7%	43 48.3%	89 100%	
Total	Count %	50 50%	50 50%	100 100%	

		Cases	Controls	Total	
Both low zinc and iron	Present %	37 94.9%	2 5.1%	39 100%	Odds ratio: 68.30 Relative risk: 4.45 p- value: <0.0001
	Absent %	13 21.3%	48 78.7%	61 100%	
Total	Count %	50 50%	50 50%	100 100%	

In the study population, if they were having both low levels of Iron and Zinc, they 68.3 times more risk for developing febrile seizures with p-value <0.0001. Relative risk is 4.45. if they were having only low levels of zinc or iron, the relative risk is 0.704 and 1.227. Both were not significant.

DISCUSSION

Febrile convulsions are one of the common paediatric emergencies encountered around the world. Febrile seizures occur in 6 months to 5 years of age[4,31]. Incidence of febrile convulsions around the world is between 3-4%. It is of similar incidence all over the world. At least 3-4 % children may have one episode below 5years of age. In India, incidence is almost 10% according to some studies. But recent studies indicate that incidence is almost comparable to western population[3]. In febrile children, some may develop febrile convulsions and some may not develop febrile convulsions. The underlying mechanism is still not clear. Various mechanisms like genetic factors, family history of febrile convulsions, deficiency of Iron and zinc were proposed[59]. Various studies showed that deficiency of Iron and zinc as risk factors for development of convulsions. In this study, we hypothesised that Low Zinc and Iron levels may lead to development of febrile convulsions.

1) **Mean age in months:**

In the present study mean age was 22.62 ± 12.45 months. There was no statistical difference between two groups (p-value >0.05). A study conducted at Chennai by Ganesh et al showed a mean age of 23.8 months[5]. Mahyar et al conducted a case control study in 2006 involving 52 febrile convulsion children and 52 normal healthy controls. This study showed a mean age of 27.13 ± 15.72 months[60]. Another study done in

2009 by Farah et al, showed mean age of 21.25± 11.53[61]. Another study done in Pakistan by Waqar Rabbani et al showed mean age of 23.97±14.45 months[59].Ihsankafadar et al conducted a study between august to November 2009. They found that median age in cases was 16 months[62]. According to previous studies, 18 months is the median age of onset. In almost 50% of the children, onset is between 12-30 months of age[4].

2) Sex:

In the study population, 54% (27/50) of the cases were male and 46% (23/50) were female. Male: female ratio was 1.17:1. Males are predominantly had febrile seizures when compared with females. A study done by Farah et al showed that 40% of the cases were female and 60% of the cases were male[61]. According to Ganesh et al, there was no male predominance[5]. Another study conducted by conducted by Mahyar et al showed that 57.7% were male and 42.3% were female[60]. According to study conducted by Al- Zwaini et al male: female ratio reported to be between 1.1:1 to 4:1[10]. Waqar Rabbani et al conducted a case control study in Pakistan in 2011. They found that 66 out of 100 children were male (66%)[59]. According to a study conducted byJun-Hwa Lee et al in Korea showed that 115 of 248 cases were female (46.4%)[63].

3) Aetiology of fever:

In the study population, 70% (28/50 cases) had acute respiratory infection followed by Acute Gastroenteritis (14%) and viral fever (14%) respectively. According to a study conducted by Margareta et al in 2010, acute respiratory infection is the predominant diagnosis. Tomoum et al found that 60% of the cases were due to viral etiology[64].Waqar Rabbani et al found that upper respiratory tract infection is the predominant etiology (24%) followed by pneumonia (16%), urinary tract infection (16%) respectively[59]. Jun-Hwa Lee et al found that acute tonsillo pharyngitis is the predominant etiology followed by viral fever, bronchitis, pneumonia etc.[63].

4) Degree of temperature as a risk of seizures:

It is clear that degree of rise of temperature may be a predisposing factor for development of seizures. Mean temperature in cases and controls were 101.61 ± 1.31° Fahrenheit and 101.17 ± 0.86°F in the present study. The difference between two groups was not statistically significant (p-value > 0.05). According to the study conducted by Ganesh et al, mean temperature in cases and controls were 102°F and 101.4°F respectively[5]. Margareta et al in 2009 found that mean temperature in cases and controls were 39.01± 0.56° C and 38.64 ± 0.45° C[64]. According to Jun-Hwa Lee et al, mean temperature in cases and controls were 38.3±0.9 °C and 36.5±0.3 ° C respectively. They found that it was

statistically significant[63]. Hassan et al conducted a case control study in Egypt found that mean temperature in cases and controls were 39±0.5 °C and 38.5±0.7° C respectively[65].

5) Family history of febrile seizures:

Family history of febrile convulsions was seen in 22% of the cases (11/50 cases). There was no family history of febrile seizures in the control group in the present study. The difference between the two groups was statistically significant (p-value <0.001). A study conducted by Margareta et al showed that family history of febrile seizures is an important risk factor[64]. According to a study conducted by Sadleir et al, 24% of febrile seizure children had family history[4]. According to karande et al done in India, 25-40% of febrile convulsion children had history of febrile convulsion in the family[3]. Hassan et al. studied 40 cases of febrile seizures and 40 cases of febrile children in Egypt. They found that 87.5% (35/40 cases) had family history of febrile convulsion[65]. Ihsankafadar et al found that 44.4% had positive h/o febrile convulsions in the family[62].

6) Role of Zinc:

In brain, zinc is present in large quantities in the hippocampus. Zinc regulates glutamic acid decarboxylase activity which is an important enzyme in production of γ- amino butyric acid. It also regulates the neurotransmitter affinity. It mediates inhibition of calcium on N-methyl-

D-aspartate receptors there by reducing excitatory discharge of neurons. In deficiency of zinc, these receptors get stimulated which may produce epileptiform discharges in children with fever[5].

Zinc also activates pyridoxal kinase, which in turn helps in the pyrioxal phosphate synthesis from pyridoxal. Pyridoxal phosphate inturn activates glutamic acid decarboxylase which involved in synthesis of GABA. Post synaptic receptors in interaction with zinc assists in GABA action. Hence hypozincemia leads to decrease in GABA level which leads to development of seizures[5].

In the present study, mean serum zinc levels in cases and controls were 49.8 ± 38.6 and 92.2 ± 33.5 μg/dl respectively. The difference between the two groups was statistically significant (p-value <0.0001). Normal Zinc levels were ranging from 65-120μg/dl. Highest value was126μg/dl. Lowest value was 14μg/dl. A study done by Ganesh et al. showed that mean Zinc levels in cases and controls were 32.17 ± 15.05 and 87.6 ± 17.6 μg/dl respectively[5]. According to Mahyar et al, mean zinc in cases and controls were 62.84 ±18.40 and85.70 ± 16.76 μg/dl respectively[60]. Waqar Rabbani et al found that low zinc levels may be a risk factor for development of febrile convulsions[59]. According to a study done by Hassan et al, median Zinc levels in cases and controls were 53 and 93μg/dl respectively. A study conducted in Turkey by

Ihsankafadar et al found that there was no statistical difference of mean Zinc levels between cases and controls (110.49±35.03 μg/dL vs 107.12±21.66 μg/dL)[62].

7) Role of iron:

Iron is an important element for metabolism in the brain. It also helps in neuro transmitter metabolism. Deficiency of iron acts as an important factor in development of febrile seizures[6]. Deficiency of iron was diagnosed by combination of following parameters like haemoglobin <11gm/dl, MCV < 70 fl, MCHC <31%, MCH <23pg, Serum iron < 65μg/dl, RDW >15%, serum ferritin <12ng/ml.

In the present study, mean Iron levels in cases and controls were 51.4 ± 37.0 and 82.3 ± 18.6 μg/dl respectively. Lowest iron level in cases was13μg/dl. Highest level in cases was 67.4μg/dl.Thedifference between the two groups was statistically significant. A study done by Pisicane et al showed that mean serum iron in the cases were significantly lower than controls[6]. According to the study done by leelakumari et al, mean Serum iron was significantly lower[6]. According to Hassan et al , median serum iron level in cases and controls was 34 and 129μg/dl respectively[65]. Fallah et al conducted a study in Iran found that serum iron levels in cases and controls were 48.91 ± 22.96 and 75.13 ± 35.57 μg/dl. According ghasem et al, mean iron level in cases and controls were

40.88±22.16 and 43.18±23.35µg/dl respectively. But the difference between two groups was not statistically significant[66].

In the present study, mean haemoglobin in cases and controls were 10.6 ± 0.9 and 11.6 ± 0.8. Lowest haemoglobin value in cases was 8.6gm/dl. Highest haemoglobin value was12.2gm/dl. The difference between the two groups were statistically significant (p-value <0.0001). Pisicane et al. found that mean haemoglobin was significantly lower when compared with controls. According to study done by Leelakumari et al, mean haemoglobin in cases and controls were 9.4±1.2 gm/dLand 9.5±1.0 g/dL respectively. There was no significant difference found in this study [6]. According to the study conducted by Hartfield et al, there was no significant difference between two groups (11.7 vs 11.6)[67].

Mean MCV levels in cases and controls were 68.7 ± 6.3 and 76.5 ± 6.5 respectively. The difference between the two groups were statistically significant (p-value <0.0001). Highest MCV value was 82.8 fl and lowest value 52.8 fl. According to Hartfield et al, mean MCV levels were significantly lower when compared with controls (76.6 vs 77.8)[67]. According to Leelakumari et al, mean MCV levels were lower but it was not statistically significant[6]. Ghasem et al conducted a study in Iran and found that mean MCV levels in cases and controls were 78.14±7.35 and 79.63±5.40fl respectively[66].

Mean MCHC levels in cases and controls were 33.1 ± 1.4 and 33.6 ± 1.1 % respectively. There was no statistical significance (p-value 0.062). Ghasem et al found that mean MCHC values were 31.46±1.76 and 31.78±1.24 % respectively[66].

Mean MCH levels in cases and controls were 22.8 ± 2.4 and 26 ± 2.6 respectively. The difference between the two groups was statistically significant (p-value <0.001). leelakumari et al found out that there were relatively low red cell indices when compared with controls[6].

Mean RDW levels in cases and controls were 16.1 ± 1.6 and 14.3 ± 1.5 respectively. The mean RDW levels were significantly higher in cases when compared with controls (p-value <0.0001). Leelakumari et al found out that Mean RDW values were significantly higher when compared with controls[6].According to Hartfield et al mean RDW levels in cases and controls were 14 and 13.9 respectively[67]. Hassan et al found that mean RDW levels in cases and controls were 16.8 ± 1.3 and 12.7 ± 1.1 respectively. The difference is statistically significant[65].

In this study we also compared between Mean levels of zinc, haemoglobin, MCV, MCHC, MCH, RDW in < 24 months and > 24 months age group separately. We found out that serum zinc levels were almost similar in both groups. There was comparatively lower mean levels of Iron, MCV, MCHC, RDW in < 24 months group than >24 months group.

Odds ratio for development of febrile seizures was more, if they were having both low Iron and Zinc levels. If they were having either low Iron or Zinc, there was no increased risk when compared with controls.

Limitations of the study:

1) It was a relatively smaller and hospital based study, a larger study is required to confirm these findings.
2) Serum ferritin levels could not be measured due to financial constraints.

CONCLUSIONS

1) Iron and zinc levels were significantly low in children with febrile seizures when compared to febrile children without seizures.
2) Serum Iron was very much low in children < 2years of age when compared to > 2years of age.
3) There is no age wise difference in mean Zinc levels.
4) There was 68.3 times increased risk of developing seizures, if they were having both low Iron and Zinc levels.

RECOMMENDATIONS

- The study supports the existing relationship between low Iron and zinc levels with febrile seizures
- Supplementation of Iron and Zinc may be helpful to prevent recurrence of febrile seizures.

BIBLIOGRAPHY

[1] Graves R, Oehler K, Tingle L. Febrile seizures: risks, evaluation, and prognosis. Am Fam Physician 2012;85:149–53.

[2] Mohammadi M. Febrile seizures: four steps algorithmic clinical approach. Iran J Pediatr 2010;20:5–15.

[3] Karande S. FEBRILE SEIZURES□: A REVIEW FOR FAMILY PHYSICIANS. Indian J Med Sci 2014;61:21–6.

[4] Sadleir LG, Scheffer IE. Febrile seizures. BMJ 2007;334:307–11.

[5] Ganesh R, Janakiraman L. Serum zinc levels in children with simple febrile seizure. Clin Pediatr (Phila) 2008;47:164–6.

[6] leelakumari P MN. Iron Deficiency as a Risk Factor for Simple Febrile Seizures –. Indian Pediatr 2012;49:5–7.

[7] Gupte S. Textbook Of Pediatric Nutrition. Peepee Publishers and Distributors (P) Limited; 2006.

[8] Hirtz DG. Febrile seizures. Pediatr Rev 1997;18:5–8.

[9] Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia 2014;55:475–82.

[10] AL-Zwaini EJ. Risk factors for a first febrile seizure: a matched case-control study. Iraqui Postgrad Med J 2006;5:13–8.

[11] Mahyar A, Ayazi P, Fallahi M, Javadi A. Risk factors of the first febrile seizures in Iranian children. Int J Pediatr 2010;2010:1–3.

[12] Vestergaard M, Basso O. Risk factors for febrile convulsions. Epidemiology 2002;13:282–7.

[13] M B. Relevance of a family history of seizures. Arch Dis Child 1983;58(6):404–5.

[14] Singhi PD, Jayshree K. FEBRILE SEIZURES□: AN UPDATE. Indian Pediatr 1980;32:564–72.

[15] Iwasaki N, Nakayama J, Hamano K, Matsui A, Arinami T. Molecular genetics of febrile seizures. Epilepsia 2002;43 Suppl 9:32–5.

[16] Johnson WG, Kugler SL, Stenroos ES, Meulener MC, Rangwalla I, Johnson TW, et al. Pedigree analysis in families with febrile seizures. Am J Med Genet 1996;61:345–52.

[17] Hedera P, Ma S, Blair M a, Taylor K a, Hamati A, Bradford Y, et al. Identification of a novel locus for febrile seizures and epilepsy on chromosome 21q22. Epilepsia 2006;47:1622–8.

[18] Wallace R, Berkovic S. Suggestion of a major gene for familial febrile convulsions mapping to 8q13-21. J Med Genet 1996;33:308–12.

[19] Johnson EW, Dubovsky J, Rich SS, O'Donovan C a, Orr HT, Anderson VE, et al. Evidence for a novel gene for familial febrile convulsions, FEB2, linked to chromosome 19p in an extended family from the Midwest. Hum Mol Genet 1998;7:63–7.

[20] Hak E, Bonten M. MMR vaccination and febrile seizures. JAMA 2004;292:351–7.

[21] Barlow W, Davis R. THE RISK OF SEIZURES AFTER RECEIPT OF WHOLE-CELL PERTUSSIS. N Engl J Med 2001;345:656–61.

[22] Centers for Disease Control and Prevention. Update: recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010-11. MMWR Morb Mortal Wkly Rep 2010;59:989–92.

[23] Chung B, Wong V. Relationship between five common viruses and febrile seizure in children. Arch Dis Child 2007;92:589–93.

[24] Reid AY, Galic M a, Teskey GC, Pittman QJ. Febrile seizures: current views and investigations. Can J Neurol Sci 2009;36:679–86.

[25] Heida JG, Moshé SL, Pittman QJ. The role of interleukin-1β in febrile seizures. Brain Dev 2010;31:388–93.

[26] Schuchmann S, Schmitz D, Rivera C, Vanhatalo S, Salmen B, Mackie K, et al. Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. NAt Med 2007;12:817–23.

[27] Village EG. Neurodiagnostic evaluation of the child with a simple febrile seizure. Pediatrics 2011;127:389–94.

[28] Capovilla G, Mastrangelo M, Romeo A, Vigevano F. SUPPLEMENT – ITALIAN LEAGUE AGAINST EPILEPSY Recommendations for the management of “‘ febrile seizures ’” Ad hoc Task Force of LICE Guidelines Commission. Epilepsia 2009;50:2–6.

[29] Shinnar S, Chan S, Hesdorffer DC, Lewis D V, Macfall J, Pellock JM, et al. MRI abnormalities following febrile status epilepticus in children The FEBSTAT study. Neurology 2012;79:871–7.

[30] Baumann RJ, Overview A. Technical Report : Treatment of the Child With Simple Febrile Seizures. AAP 1999.

[31] Village EG. Febrile Seizures□: Clinical Practice Guideline for the Long-term Management of the Child With Simple. AAP 2008.

[32] Rose W, Kirubakaran C, Scott JX. Intermittent clobazam therapy in febrile seizures. Indian J Pediatr 2005;72:31–3.

[33] Vestergaard M, Pedersen MG, Østergaard JR, Pedersen CB, Olsen J, Christensen J. Death in children with febrile seizures□: a population-based n.d.:457–63.

[34] Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. J Res Med Sci 2013;18:144–57.

[35] Deshpande J, Joshi M, Giri P. Zinc: The trace element of major importance in human nutrition and health. Int J Med Sci Public Heal 2013;2:1.

[36] Nriagu J. Zinc toxicity in humans. Encycl Environ Heal 2007:1–7.

[37] Sekler I, Sensi S. Mechanism and regulation of cellular zinc transport. MOL MED 2007;13:337–43.

[38] King JC, Shames DM, Woodhouse LR. Zinc and Health□: Current Status and Future Directions Zinc Homeostasis in Humans. J Nutr 2000:1360–6.

[39] Lo B. Zinc and Health : Current Status and Future Directions Dietary Factors Influencing Zinc Absorption 1. J Nutr 2000:1378–83.

[40] who. Vitamin and mineral requirements in human nutrition Second edition. WHO, 2004.

[41] Debjit Bhowmik C. A potential medicinal importance of zinc in human health and chronic diseases. Int J Pharm Biomed Sci 2010;1:5–11.

[42] Nriagu J. Zinc deficiency in human health. Encycl Environ Heal 2010:1–8.

[43] hiroyuki yanagisawa. Zinc Deficiency and Clinical Practice. J Japan Med Assoc 2004;129:359–64.

[44] Lukacik M, Thomas RL, Aranda J V. A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. Pediatrics 2008;121:326–36.

[45] Bhandari N, Bahl R, Taneja S, Strand T, Mølbak K, Ulvik RJ. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomised controlled trial in an urban slum. BMJ 2002;324:1–5.

[46] Brooks WA, Yunus M, Santosham M, Wahed MA, Nahar K, Yeasmin S, et al. Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial. The Lancet 2004;363:1683–8.

[47] Shankar a H, Genton B, Baisor M, Paino J, Tamja S, Adiguma T, et al. The influence of zinc supplementation on morbidity due to Plasmodium falciparum: a randomized trial in preschool children in Papua New Guinea. Am J Trop Med Hyg 2000;62:663–9.

[48] Müller O, Becher H, Zweeden AB Van, Ye Y, Diallo DA. Effect of zinc supplementation on malaria and other. BMJ 2001;322:1–6.

[49] GROUP TZS. Effect of zinc on the treatment of Plasmodium falciparum malaria in. Am Jounal Clin Nutr 2002;76:805–12.

[50] Baqui AH, Black RE, Arifeen S El, Yunus M, Chakraborty J, Ahmed S, et al. Effect of zinc supplementation started during diarrhoea on morbidity and mortality in Bangladeshi children: community randomised trial. BMJ 2002;325:1–7.

[51] Sazawal S, Black RE, Menon VP, Dinghra P, Caulfield LE, Dhingra U, et al. Zinc Supplementation in Infants Born Small for Gestational Age Reduces Mortality: A Prospective, Randomized, Controlled Trial. Pediatr 2001;108 :1280–6.

[52] Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro R a, Weill R, Boccio JR. The role of zinc in the growth and development of children. Nutrition 2002;18:510–9.

[53] Ehsanipour F, Taher MT. Serum Zinc Level in Children with Febrile Convulsion and its Comparison with that of Control Group. Iran J Pediatr 2009;19:65–8.

[54] Füessl HS. [Iron deficiency anaemia]. MMW Fortschr. Med., vol. 156, 2014, p. 1–72.

[55] Goddard AF, James MW, McIntyre AS, Scott BB. Guidelines for the management of iron deficiency anaemia. Gut 2011;60:1309–16.

[56] Baker RD, Greer FR. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics 2010;126:1040–50.

[57] Johnston M V. Iron deficiency, febrile seizures and brain development. Indian Pediatr 2012;49:13–4.

[58] Kliegman RM, Stanton BMD, Geme JS, Schor NF, Behrman RE. Nelson Textbook of Pediatrics, Elsevier Health Sciences; 2011.

[59] Waqar Rabbani M, Ali I, Zahid Latif H, Basit A, Rabbani MA. Serum Zinc Level in Children Presenting with Febrile Seizures. Pakistan J Med Sci 2013;29:1008–11.

[60] Mahyar A. SERUM ZINC LEVEL IN CHILDREN WITH FEBRILE SEIZURE. Acta Med Iran 2008;46:477–80.

[61] Farah A, Ahmad G. serum zinc level in patients with simple febrile seizures. Iran J Child Neurol 2010;4:41–4.

[62] Kafadar I, Akinci AB, Pekun F, Adal E. The Role of Serum Zinc Level in Febrile Convulsion Etiology. Çocuk Enfeksiyon Dergisi/Journal Pediatr Infect 2012;6:90–3.

[63] Lee J-H, Kim JH. Comparison of serum zinc levels measured by inductively coupled plasma mass spectrometry in preschool children with febrile and afebrile seizures. Ann Lab Med 2012;32:190–3.

[64] Margaretha L, Masloman N. correlation between serum zinc level and simple febrile seizure in children. Paediatr Indones 2010;50:326–30.

[65] Hassan IAERMAHMKDRS and M, Mohamed. Iron profile parameters and serum zinc & copper levels in children with febrile convulsions in Banha. J Am Sci 2014;10:1–5.

[66] Miri-aliabad G, Khajeh A, Arefi M, Health A, Seizure F, Deficiency I. Zahedan Journal of Research in Medical Sciences 2013.

[67] Hartfield DS, Tan J, Yager JY, Rosychuk RJ, Spady D, Haines C, et al. The association between iron deficiency and febrile seizures in childhood. Clin Pediatr (Phila) 2009;48:420–6.

ANNEXURES

ABBREVATIONS

AAP	-	American Academy of Paediatrics
ILAE	-	International League against Epilepsy
NIH	-	National Institute of Health
NICU	-	Neonatal Intensive Care Unit
GEFS+	-	Generalised Epilepsy Febrile Seizures Plus
MMR	-	Measles, Mumps, Rubella
DPT	-	Diphtheria, Pertussis, Tetanus
TIV	-	Trivalent Influenza Vaccine
LAIV	-	Live Attenuated Influenza Vaccine
ACIP	-	Advisory Committee on Immunisation practices
HHV-6	-	Human Herpes Virus 6 infection
RSV	-	Respiratory syncytial Virus
GABA	-	Gamma-Amino Butyric Acid
IL-1β	-	Interleukin 1β
NMDA	-	N-Methyl-D-Aspartate receptor
EEG	-	Electro Encephalogram

CNS	-	Central Nervous System
CSF	-	Cerebro Spinal Fluid
Hb	-	Haemoglobin
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
RDW	-	Red cell Distribution Width
WHO	-	World Health Organization

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INSTITUTIONAL HUMAN ETHICS COMMITTEE RESEARCH, COIMBATORE

PSG INSTITUTE OF MEDICAL SCIENCES AND PARENTAL CONSENT FORM

Your (son/daughter/child) is invited to participate in a study of estimation of serum zinc and iron levels in children with simple febrile seizures.

My name is Dr.KodaliKirti Chandra and I am a postgraduate at PSGIMS&R, Coimbatore.

I am asking for permission to include your (son/daughter/child) in this study because febrile seizures are one of most common causes of hospitalization in children between 6 months to 5 years. Adequate levels of zinc and iron usually help in prevention of seizures. With this study zinc and iron levels will be estimated, so that can be helpful in reduction of febrile seizures by zinc and iron supplementation. I expected to have 100 participants in the study. If you allow your child to participate I will take 2ml blood for zinc and iron estimation.

Any information that is obtained in connection with this study and that can be identified with your (son/daughter/child) will remain confidential and will be disclosed only with your permission. His or her responses will not be linked to his or her name or your name or your name in any written or verbal report of this research project.

Your decision to allow your (son/daughter/child) to participate will not affect your or his or her present or future relationship with PSGIMS&R or PSG HOSPITALS. if you have any questions about the study, please ask me. If you have any questions later, call me at 9790283554. If you have any questions or concerns about your (son/daughter/child)'s participation in this study call 9790283554.

You may keep a copy of this consent form.

You are making a decision about allowing your (son/daughter/child) to participate in this study. your signature below indicates that you have read the information provided above and have decided to allow him or her to participate in the study. If you later decide that you wish to withdraw your permission for your (son/daughter/child) to participate in the study, simply tell me.

You may discontinue his or her participation at any time. This will not affect in any way your future treatment in this hospital.

Name of (son/daughter/child):

Signature of parent or legal guardian

Signature of investigator

Date:

Date:

IHEC CONTACT NUMBER: +91-0422-2570170

உங்கள் (மகன்/மகள்/குழந்தை)யை என் ஆய்விற்கு (ஆராய்ச்சிக்கு) பங்கேற்க வேண்டி

ஒப்புதல் கடிதம்

கோடாளிகிரிசந்திரா ஆகிய நான் மு.குழந்தைகள் பிரிவில் மேற்படிப்பிற்காக முதாலாமண்டு பி..எஸ்.ஜி மருத்துவ கல்லூரியில் படித்துக் கொண்டு இருக்கிறேன்.

நான் உங்கள் (குழந்தை/மகன்/மகள்)-யை இந்த ஆய்விற்கு (ஆராய்ச்சிக்கு) பங்கேற்று கொள்ள சம்மதம் கேட்டுக்கொள்கிறேன். ஏனென்றால் இவ்வாய்விற்கு (ஆராய்ச்சிக்கு) 100 பேர்கள் தேவைப்படுகிறார்கள்.

இதன் ஆய்வில் இருந்து பெறப்படும் மகன்/மகள்/குழந்தைகள் பற்றிய தகவல்கள் அனைத்தும் பாதுகாப்பாக வைக்கப்படும் என்றும் எந்த நேரத்திலும் மற்றவர்களிடம் தெரியப்படுத்தமாட்டேன் என்பதையும் தெரிவித்துக் கொள்கிறேன். உங்கள் விருப்பத்துடன் மட்டும் அந்த தகவல்கள் வெளிப்படுத்தப்படும் என்பதையும் தெரிவித்துக் கொள்கிறேன்.

இந்த ஆய்வில் பங்கேற்பதால் உங்களுக்கோ அல்லது உங்கள் குழந்தைக்கோ அளிக்கப்படும் சிகிச்சைகளில் எந்தவித மாற்றமும் இருக்காது. உங்களுக்கு ஏதேனும் இவ்வாராய்ச்சிப் பற்றிய சந்தேகங்கள் இருந்தால் என்னிடம் கேட்டுக் கொள்ளலாம். நீங்கள் என்னை தொலைபேசி மூலம் (தொலைப்பேசி எண் -9790283554) தொடர்புக் கொள்ளலாம்.

இந்த ஒப்புதல் கடிதத்தின் நகலை நீங்களும் ஒன்று வைத்துக் கொள்ளலாம். இதன்மூலம் நீங்கள் உங்கள் குழந்தை/மகன்/மகளை என் ஆய்வில் பங்கேற்க ஒப்புக்கொண்டீர்கள் என்றும், கீழே போடப்பட்டுள்ள உங்கள் கையொப்பம் மேற்கூறிய அனைத்தையும் நீங்கள் படித்துப் பார்த்து, நன்கு அறிந்த பின்னரே சம்மதம் கொடுத்தீர்கள் என்பதைக் குறிக்கின்றன.

மேலும் நீங்கள் இந்த ஆய்விலியிருந்து பின்பு விலகி கொள்ள விரும்பினால் என்னிடம் தெரியப்படுத்த வேண்டும் என்பதை கேட்டுக்கொள்கிறேன். எந்த நேரத்திலும் உங்க விருப்பத்தின்படி விலகிக் கொள்ளும் உரிமை உங்களுக்கு இருக்கு. இதனால் உங்களுக்கு அளிக்கப்படும் சிகிச்சைகளில் எந்தவித மாற்றமும் இருப்பதில்லை என்பதையும் தெரிவித்துக் கொள்கிறேன்.

-குழந்தை/மகன்/மகளின் பெயர்

பெற்றோர் கையொப்பம்

ஆய்வு மேற்கொள்பவரின்

கையொப்பம்

PROFORMA

Name:

Age:

Sex:

Barcode number:

Diagnosis:

Weight:

Recent history of immunisation:

Family history of febrile seizures:

Temp:

Duration of fever:

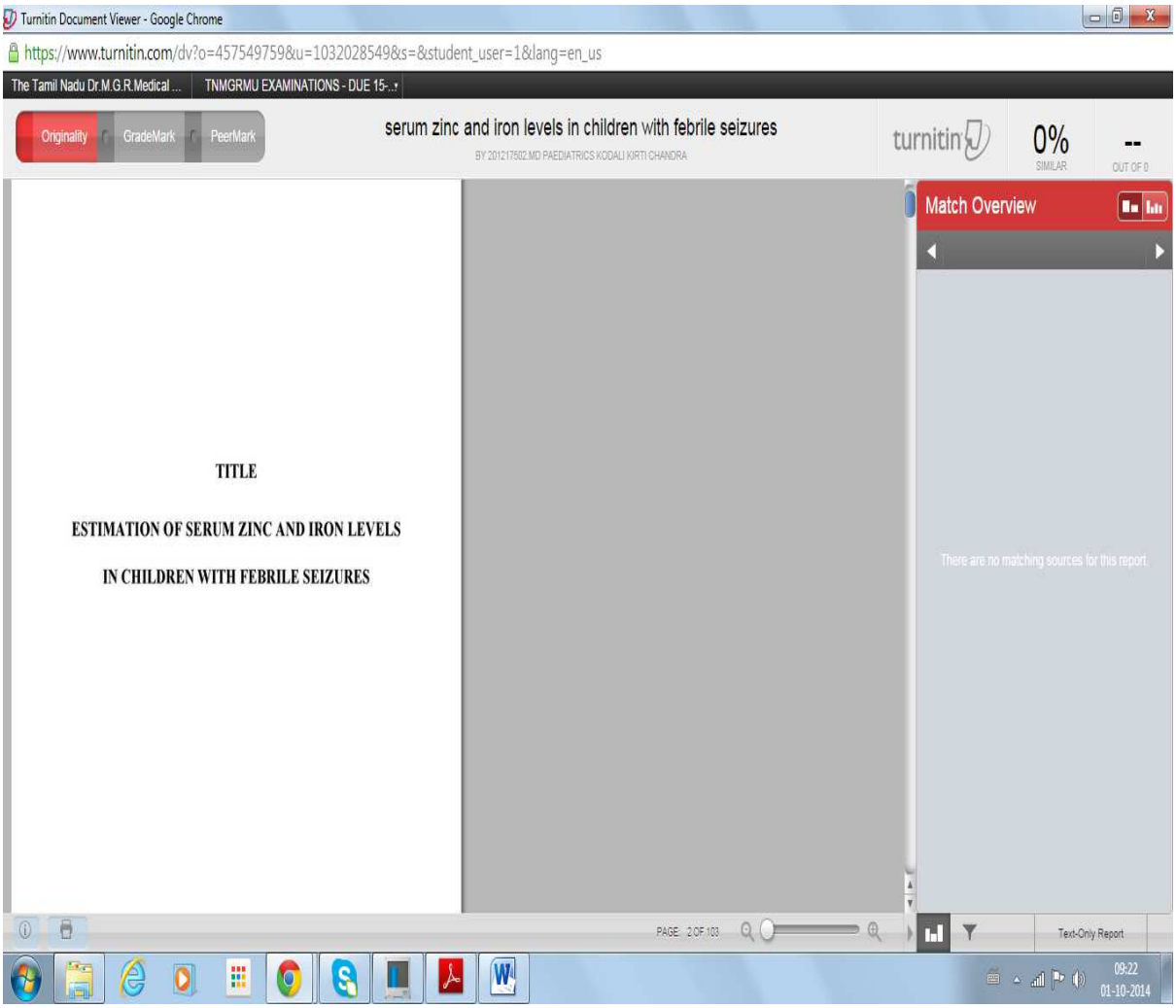
Duration of seizures: <5 min /5-10min/10-15 min

Total Monthly family Income:

Education:

Occupation:

PLIAGARISM CLEARANCE



MASTER CHARTS

NAME	IP.NO	DIAGNOSIS	RECURRENCE	CASE/ CONTROL	AGE IN MONTHS	SEX	TEMP	RECENT IMMUNISATION	FAMILY HISTORY	F-S INTERVAL (HRS)	DURATION OF SEIZURE	SOCIO ECONOMIC STATUS	WEIGHT IN KG	NUTRITION STATUS	SR.ZINC	SR.IRON	HB	MCV	MCHC	MCH	RDW
VISHNU SRIRAM	13/20003	1	N	1	11	1	104	NO	2	18	1	4	9.8	1	24.6	31	11	69.4	32.3	22.5	15.4
SUBHASH	13/20180	1	Y	1	24	1	100.8	NO	1	14	1	5	10	1	74.86	51.3	9	68.8	32	21.8	15.3
THIRUCHITRAMBALA EASAN	13/20604	4	N	1	12	1	101.6	NO	2	8	2	3	8.6	1	18	61.2	10.9	60.4	31.5	19	15.8
B/O SRISATHYA	13/20713	3	NA	2	12	1	101.1	NO	2	NA	NA	3	11	1	22.38	71.8	12.2	80	34	27.2	13.4
AMUTHA'S BABY	13/20722	3	N	1	14	1	100.2	NO	2	10	1	5	9.1	1	34.5	43	10	72.6	32.5	23.6	16.5
VINOTHINI'S BABY	13/21335	4	N	1	16	2	102	NO	2	14	1	5	8.9	1	156.2	128	11.9	81.2	33.4	27.1	13.4
PRANESH	13/21419	2	N	1	12	1	100	NO	2	15	2	4	10.3	1	28.4	19.4	10.3	62.3	31	19.3	16.7
JESURAN	13/21758	3	N	1	18	1	104	NO	2	12	1	3	9.8	1	20.38	49.4	10.5	66	31.5	20.8	16.1
SHANTHINI	O13052158	1	Y	1	24	2	102	NO	1	16	1	3	9.6	2	59.2	58.1	9.6	65	30.4	20.6	16.2
YUGITHA	13/22703	1	Y	1	30	2	101.5	NO	1	20	2	4	11.5	1	28.2	34.2	10.6	64.5	32.6	22.9	16.3
SHARMILA BANU'S BABY	13/22939	3	N	1	18	1	100.6	NO	2	22	1	5	8.7	2	41.4	53.88	10.9	82.2	32.3	26.5	15.8
SATHYAPRASAD	13/23047	1	Y	1	60	1	100.8	NO	1	12	1	2	20	1	141.9	66	14.2	83.5	32.1	26.8	12.8
RAGHAVENDRA	13/23254	1	Y	1	18	1	100	NO	2	14	2	5	9.8	1	56.2	34.3	11	68	32.9	24.3	15.1
TAMILARASI	13/23760	4	N	1	10	2	100	NO	1	22	1	2	8.5	1	58.5	156	10.9	82.8	32.9	27.3	13.4
B/O GOWRI	13/24396	3	N	1	20	2	100.6	NO	1	20	2	2	11	1	56.2	19	11	69	34.7	24.3	15.4
VIGHASE	13/24822	1	Y	1	18	1	101.2	NO	2	12	2	5	9	1	156	42	8.7	67.9	32.3	21.9	20.8
MAHESWARI'S BABY	13/24846	1	N	1	6	1	100.6	NO	1	14	1	5	7	1	52.6	51	10.2	67.3	31.2	22.2	18.1
SATHISH	13/24831	3	Y	1	48	1	101	NO	2	10	1	4	15	1	22.56	34	10.1	66.9	32.5	21	16.7
SAMVEL SATHYA	13/24954	1	Y	1	24	1	102	NO	2	22	1	5	12	1	26.5	19	10.9	66.3	32.7	21.7	17.5
SAADHANA	13/25129	1	N	1	48	2	100	NO	1	10	1	5	12.4	2	138.4	186	12.1	81.6	32.4	26.4	13.9
B/O SELVI	13/25273	1	N	1	10	1	104	NO	2	14	1	5	7.7	1	91.8	13	10.8	68	32.6	24.9	15.1
CHITRA'S BABY	13/26304	1	N	1	24	2	100.6	NO	2	12	1	4	9	2	22.6	53	11	69	31.9	24.2	15.2
PRIYA'S BABY	13/27095	1	N	1	18	2	103	NO	2	14	1	5	14	1	51	17	10.5	67	30.8	24.3	16.2
JINIYA	13/28986	1	N	1	24	2	102	NO	2	16	1	2	9.5	2	31.6	57.12	10.2	68.6	34	21.5	17.4
KIRISHIKA	13/31099	1	N	1	11	2	101	NO	1	12	1	2	7.6	2	116.1	52	11	69	35.8	25.6	15.4
B/O RAJESWARI	13/32419	4	Y	1	12	2	104	NO	1	14	2	5	11	1	49.5	16	10.8	68.6	34	25.4	15.6
B/O JAYANTHI	13/33217	1	N	1	18	1	101	NO	2	16	1	5	8.5	2	128	19.4	10.4	69.7	31.2	21.8	17.7

SANTHOSH.V	13/33919	4	Y	1	36	1	103	NO	2	16	1	4	12.7	1	56.5	124	12.1	79.3	31.1	24.7	15
SRIVIKASH	13/34543	2	N	1	24	1	103	NO	2	14	2	4	11.1	1	14	22	9	60.4	32.8	19.8	19.8
SREERAM	13/36423	2	NA	2	6	1	100	NO	2	NA	NA	5	11	1	111.4	98	11.5	75.2	36	27.8	14.3
ASHWIN	13/36904	2	N	1	24	1	104	NO	2	14	1	5	12.5	1	57.6	30.7	10.7	68.2	32.3	21.1	16.8
PREETHI	13/37305	2	NA	2	14	2	102	NO	2	NA	NA	5	7.4	2	45.33	57	10.2	66.1	33.5	22.1	17.1
SANTHOSH.T	13/37666	4	NA	2	60	1	104	NO	2	NA	NA	4	16	1	120.5	82.9	11.6	75.2	34.2	28.5	13.4
RANJANI	13/37680	2	N	1	18	2	101.2	NO	2	14	1	4	10	1	90	49.6	10.2	69.3	34.5	23.7	15.6
DHARUN AKASH	13/37751	4	NA	2	48	1	102	NO	2	NA	NA	5	15.6	1	98.2	87.6	11.2	78.3	33.1	25.9	13.8
BHAVANIKA	13/37761	3	NA	2	22	2	101.2	NO	2	NA	NA	5	9.3	2	97.1	58.7	10.5	63.2	31.7	20	16.2
KALPANA'S BABY	13/38668	1	N	1	12	2	102.5	NO	2	12	1	5	9.5	1	23.38	29.7	10.6	69.1	33.7	23.3	17.9
VIJI'S BABY	14/00234	2	NA	2	24	2	101	NO	2	NA	NA	4	9.4	2	123.5	46	9.6	58.5	32.8	19.2	19.7
JAYASHREE'S BABY	14/00478	1	N	1	36	1	104	NO	2	12	2	2	14	1	24.5	78	12.1	76.7	34.2	26.2	13.2
SALVIN KIRBA	14/00652	2	NA	2	10	1	103	NO	2	NA	NA	5	7.9	1	102.8	89.2	11.7	79.3	32.1	27.2	13.3
MAHASRI	14/00291	4	NA	2	10	1	102	NO	2	NA	NA	4	10	1	98.3	87.5	11.6	77.8	33.5	28.5	14.3
ARYA RANJITH	14/00643	1	N	1	24	1	103	NO	2	10	1	2	12.5	1	57.6	20	10.2	68.6	33	22.6	19.3
ANUSHREE	O13037625	3	Y	1	12	2	103	NO	2	8	1	5	10.5	1	26.8	46.6	10.3	66	32	18.8	18.1
SHARUMATHI	14/09294	1	N	1	15	2	100.4	NO	2	10	2	5	11	1	16	27	10.9	63.6	32.7	20.8	16.6
ANTHONY STELLA'S BABY	14/09955	4	N	1	11	1	101	NO	2	12	1	5	8	1	44.3	153.4	11.9	74.3	32.1	23.9	14.4
POORNIMA'S BABY	14/12633	4	NA	2	6	1	100.2	NO	2	NA	NA	4	7	1	79.05	90.5	11.6	72.6	33.4	24.2	13
HASHINI	14/12756	1	Y	1	36	2	100	NO	2	4	1	2	10.5	2	22.4	56.4	10.6	52.8	34.7	18.2	18.3
DAKSHATHA	14/13001	4	Y	1	18	2	100.8	NO	2	12	1	5	10.2	1	47.6	48.5	9.6	62.4	35	20.3	16.1
MADHAV	14/13068	3	NA	2	36	1	101	NO	2	NA	NA	2	10.2	2	152.6	87.5	11.6	81.8	34.3	28	13.7
SARSHANA	14/14628	1	N	1	12	2	101	NO	2	12	1	2	10	1	25.6	53.7	10.4	63.3	34.5	20.2	16.7
VIDHYUTHA	14/14640	1	N	1	12	2	102	NO	2	14	1	1	8	1	18.79	45.56	9.1	55.3	34.3	19	16.9
SHANTHI'S BABY	14/16027	1	N	1	36	1	101	NO	2	4	1	5	15.8	1	24.5	34.46	10.8	69	35.8	19.8	15.4
SHITTESH	14/18122	4	NA	2	60	1	101.2	NO	2	NA	NA	5	18.8	1	201.4	111.5	11.3	81.5	34.6	28.2	13.1
NEELAVATHI'S BABY	14/18335	1	N	1	24	1	100.6	NO	2	4	1	5	10.7	1	16.07	56.5	10.8	68.6	35.2	25.3	15.2
ASHWATHI	14/18406	3	NA	2	48	2	100	NO	2	NA	NA	2	13.6	1	78.2	87.5	11.3	79.5	34.3	27.3	13.7
ASHISH YUVIN	14/18476	2	NA	2	36	1	100.8	NO	2	NA	NA	1	14.6	1	94	87.6	12	75.5	33.8	26.5	15.1

JOHN ROBIN	14/18413	2	NA	2	36	1	101.4	NO	2	NA	NA	5	18.5	1	102	85.2	13.1	91.3	34.8	31.8	13.8
KAVIYA	14/18969	1	NA	2	18	1	101	NO	2	NA	NA	2	11	1	78.4	94	13.3	81.7	33.7	27.5	13.6
NAREN KARTHIKEYAN	14/18780	4	NA	2	22	1	101	NO	2	NA	NA	2	12	1	82.88	97.5	11.2	77.1	33.5	25.8	14.6
DEEPA'S BABY	14/18775	2	NA	2	24	1	101.4	NO	2	NA	NA	5	11.7	1	156	118	11.7	71.9	33.9	26.1	15.1
DHARAN KUMAR	14/18927	1	NA	2	8	1	101.6	NO	2	NA	NA	5	7.62	1	118	31	9.6	65.7	32.7	21.5	16.2
SHRIMATHI	14/19091	1	NA	2	28	2	100.8	NO	2	NA	NA	5	13	1	95.6	87.6	11.6	88.1	36	27.1	13.3
KALAMANI'S BABY	14/19211	3	NA	2	12	2	100.9	NO	2	NA	NA	4	8	1	145	138.4	11.9	78.2	34.2	25.3	14.2
JERON.E	14/19350	2	NA	2	8	1	100.6	NO	2	NA	NA	3	8.3	1	81.2	86	11.8	71.6	33.1	25.7	14.5
KAMALESWARAN	14/19345	3	NA	2	48	1	100.2	NO	2	NA	NA	3	13	1	82.4	87.5	12.8	78	33	25.8	14
RITHIKA	14/19645	2	NA	2	24	2	100.4	NO	2	NA	NA	4	11.6	1	94.6	102.5	12.9	79.1	34.7	27.4	14.6
HARSHIKASRI	14/19749	1	NA	2	9	2	102	NO	2	NA	NA	2	7.8	1	87.5	78.4	12.3	71.9	34.8	25.8	14.7
KAMALESH KUMARAN	14/19743	4	NA	2	24	1	100.4	NO	2	NA	NA	3	11.4	1	105	109.6	11.2	74.3	34.9	26.7	13.6
KAMALASH	14/20146	1	Y	1	48	1	101.4	NO	2	4	1	3	14.5	1	58.2	42.3	10.8	69.7	34.8	24.3	16.3
SREE VIKASH	14/20135	2	Y	1	36	1	100.6	NO	2	6	1	4	14.5	1	34.3	41.4	10.6	68.3	34.3	23.3	15.9
BENAZIR PARVEEN	14/20196	3	NA	2	8	2	101	NO	2	NA	NA	5	6.16	2	77.5	72.55	11.4	78.4	35.4	27.4	13.4
GUHAN	14/20275	4	NA	2	27	1	101.6	NO	2	NA	NA	5	13	1	74.86	72.9	11.7	81	35.5	28.7	13.3
REDHU	14/20338	1	N	1	18	2	100	NO	2	10	1	4	10	1	16.22	23	10.6	63.5	35.3	22.3	16.3
RANJITH PRASATH	14/20352	2	Y	1	36	1	100.6	NO	2	4	1	3	15	1	42.15	58.92	10.9	68	35.5	23.4	15.5
B/O NITHYA	14/20533	1	NA	2	6	1	100.2	NO	2	NA	NA	4	6.4	1	101.5	73.5	11.7	92.5	34.3	31.7	14.3
USHA'S BABY	14/20586	1	N	1	26	2	102	NO	2	20	1	5	11	1	36.8	47.6	10.5	65.5	33.6	24.2	15.3
VISHWA	14/20331	2	NA	2	11	1	100	NO	2	NA	NA	3	7.5	2	130.8	89.84	11.9	72.3	33.1	25.3	14.8
SHOBHA'S BABY	14/20574	1	NA	2	24	1	100.4	NO	2	NA	NA	2	13	1	141.9	79.5	12.5	79.3	33.4	26.5	13.1
NAKSHATRA	14/20751	5	NA	2	12	2	101.2	NO	2	NA	NA	4	11	1	103.5	84.06	12.3	78.3	35.5	26.7	13.2
SASTIKA	14/20781	3	NA	2	12	2	101	NO	2	NA	NA	4	10.5	1	106.6	62.5	9.5	63.4	31.3	19.9	18.8
SARAN	14/20687	4	NA	2	48	1	100.6	NO	2	NA	NA	5	15.5	1	109.5	72.8	12.5	76.1	32.6	27.5	14.5
KAMALIKA	14/21010	3	Y	1	48	2	104	NO	2	4	1	5	13	1	15.4	23	8.6	67	32	22.2	16.4
DHILIP	14/21077	4	NA	2	36	1	101	NO	2	NA	NA	5	14	1	116.2	92.6	12.8	79.6	33	26.3	14
ANCHANAAKSHI	14/21171	5	N	1	11	2	102.5	NO	1	4	1	4	7.5	2	19.9	55.8	10.8	69.3	34.2	22.9	15.9
PRASANNA	14/21335	2	NA	2	28	1	100.5	NO	2	NA	NA	4	12.3	1	96.7	110.8	12	82.9	32.6	27	13.7

B/O MEENAKSHI	14/22533	2	Y	1	10	1	100.3	NO	2	10	1	5	7.7	2	18.5	36.2	11	69.4	33.6	23.7	14.6
B/O SAHANA	14/22287	4	NA	2	18	1	101.2	NO	2	NA	NA	5	9.3	1	40.5	67	11.8	73.4	33.3	25.5	12.5
PRAJESH	14/22543	4	NA	2	26	1	101.2	NO	2	NA	NA	5	11	2	75	69	12.1	77.6	33.7	26.2	14.2
PRIYA'S BABY	14/23879	2	NA	2	6	1	101	NO	2	NA	NA	4	7.5	1	58.6	98.4	11.7	71.6	33.9	23.3	13.4
MANOJ KUMAR	14/23890	2	NA	2	60	1	102	NO	2	NA	NA	4	18	1	56.8	67.4	11.4	78.4	33.7	26.4	12.3
RITHIK	14/24063	1	NA	2	18	1	102	NO	2	NA	NA	3	9.4	1	46.8	56.8	10.7	68.8	30.5	21.3	16.7
RENUGA'S BABY	14/24524	1	NA	2	36	2	101.2	NO	2	NA	NA	5	10.7	2	92.6	88.8	11.4	81.7	33.3	27.3	12.7
SUMATHI'S BABY	14/24700	3	NA	2	11	1	102.4	NO	2	NA	NA	4	9	1	78.6	72.8	12.1	84.9	33.3	28.3	14.4
PRADEEPA	14/24538	2	NA	2	36	2	100.8	NO	2	NA	NA	5	12.5	1	49.2	71.28	11.7	77.3	32	26.5	13.4
NAKSHATRA	14/25439	3	NA	2	12	2	103.4	NO	2	NA	NA	4	11.5	1	40.08	75.9	11.5	72.3	33.2	24	12.7
KALAISELVI'S BABY	14/25462	2	NA	2	6	2	100.1	NO	2	NA	NA	3	5.75	2	86.2	92.6	11.4	78.6	32.9	25.8	14.2
RUTHITA.R	14/25357	2	NA	2	9	2	102	NO	2	NA	NA	4	7.1	1	78.5	68.5	11.9	75.5	32.6	24.6	15.5
FAHIMA FARZEEN	14/25285	2	NA	2	24	2	101	NO	2	NA	NA	5	11.9	1	46.8	66.5	11.5	76.5	32.8	23.8	16.5
MOHAMMED ALTHAF	14/25222	2	NA	2	24	1	100.1	NO	2	NA	NA	5	9.2	2	86.5	80.6	11.4	69.8	33	26.8	15.8
AMSALATHA'S BABY	14/25573	1	NA	2	6	1	101.4	NO	2	NA	NA	5	7.2	1	60.1	67.3	11.5	79.8	33.3	26.6	13.3